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VOLUME XVI AUGUST, 1929 No. 3

THE MOSAIC DISEASE OF THE HOP;

GRAFTING EXPERIMENTS, II

BY D. MACKENZIE, E. S. SALMON, W. M. WARE AND R. WILLIAMS.

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THE mosaic disease of the hop was first recorded in this country in 1923 when the main features were described and illustrated (6). Thrupp, in 1927 (12), showed that the disease could be transmitted by means of grafting a susceptible healthy scion on an affected stock, and also that a certain Seedling Variety of hop (ref. no. M 45), raised at Wye College, acted as a "carrier," transmitting the disease to scions of susceptible varieties while not itself being visibly affected. Salmon and Ware have pointed out (11) that the mere contact of a virus-carrying scion with the stock is sufficient to cause the infection of the rootstock; also that the commercial variety Fuggles is completely resistant to the mosaic disease, so that when scions are grafted on a severely affected hop plant, they remain healthy, grow with normal vigour and complete their full seasonal development.

I. THE PROPENSITY FOR "CARRYING" MOSAIC DISEASE, AMONGST COMMERCIAL AND OTHER VARIETIES OF THE HOP.

Methods of experiment.

The grafting experiments were carried out by D. Mackenzie[1] in 1927 and by R. Williams[1] in 1928. The general method of grafting used was that described and figured by Salmon and Ware in 1925 (7) and 1928 (10). The following details of the experiments may be mentioned.

1927 (D. Mackenzie). The scions used were about 4 to 6 in. in length and were cut at an internode near the ground level. The lower end of the scion was tapered by two downward cuts about half-an-inch long from just below a node. A corresponding cleft was made by two converging cuts downwards in the stem of the stock plant. The graft, after insertion of the scion, was bound by means of a strip of thin rubber, half-an-inch wide and 5 to 6 in. long, the end being secured by a trace of rubber solution.

[1] This work was carried out with the aid of grants from the Ministry of Agriculture and Fisheries.

The scions were taken either from plants of the commercial varieties Tutsham, Cobbs and Eastwell Golding, all of which are very susceptible to mosaic disease, or from wild hops the susceptibility or resistance of which was unknown. The plants providing scions were growing in pots, with the exception of one plant of Tutsham (R 1/50 *a*) and one of Cobbs (R 1/36 *a*) and three plants of Rodmersham Golding (R 1/81 *a*, R 1/82 *a*, R 1/83 *a*) growing in the Experimental Hop Garden at Wye. Each pot plant was labelled and the reference numbers are given in Table I. All plants from which scions were taken were kept under observation during both 1927 and 1928. During 1927 four pot plants of the Tutsham variety showed symptoms of the mosaic disease, and together with the scions taken, were discarded. Three other pot plants of Tutsham, although never showing definite symptoms of mosaic disease, were regarded as being possibly not healthy and together with the scions taken from them were discarded. The remaining plants, both in the pots (numbering 64 and in addition 27 not used) and in the hop garden (5 plants used and named above), have shown no signs of mosaic disease. The plants in pots were about a quarter of a mile distant from the stock plants used for grafting, which were all in the Experimental Hop Garden. Usually four scions were cut at a time and the greatest length of time from the taking of these to the insertion of the last was about an hour. Between each cut the razor or knife was dipped in absolute alcohol. The grafts were covered with lamp-glasses plugged with cotton wool and pressed well into the ground. Unless the soil was quite moist, it was found advisable to water the grafted plant at least once, and when the weather was dry to continue watering until the scion commenced to grow. In this process the water was kept as much as possible from touching the scion.

Between April 13th and June 3rd, 328 grafts were made; of these 216, or 65·9 per cent., were successful; the remaining 112 grafts either died or showed such weak growth that no results could be obtained from them. On June 9th the first appearance of mosaic disease on a scion was noticed.

In Table I the particulars are given of the grafting experiments in 1927. Section (*a*) contains a list of a number of different commercial varieties of hops (German, American, Danish and English) and also of Seedling and Male Hops, on all of which the scions developed mosaic disease. The symptoms appeared in all cases in unmistakable and characteristic form, evidenced by the curling and brittleness of the leaves, the mosaic mottling of the leaf tissue and cessation of growth of the stem, with the tip failing to climb and falling away from the string. Occasionally the tip of the stem wilted and died. Section (*b*) records the scions which remained healthy.

1928 (R. Williams). The method of grafting adopted was the same; in the earlier experiments the razor used in the preparation of scion and stock was kept in formalin (full strength) for some time previous to making the cuts and wiped with cotton wool before use. It was noticed afterwards that the blade still smelt strongly of formalin, but it was not until some hundred grafts had been made that it was realised that the traces of formalin affected the graft and caused the death of the scions. The method then adopted was as follows; two scalpels and two razors were kept immersed in absolute alcohol and were used alternately so that each one had been dipped in the alcohol for at least ten minutes before use. Fresh cotton wool was used each time to wipe the instruments dry. The grafting carried out from April 25th to the beginning of June, using this method of sterilisation, was successful, a fairly

high percentage of the grafts growing strongly although the cold wet spells of weather in the spring were unfavourable and slugs proved troublesome. The scions used were of the variety Eastwell Golding obtained from the farm of Mr Alfred Amos, at Wye. The "hills" from which the scions were taken were kept under observation and were free from mosaic disease throughout the season. A few grafts were also made with scions of the variety Tutsham and Rodmersham Golding. The Eastwell Golding scions were conveyed from Mr Amos' farm to the Experimental Hop Garden, two miles away, in a vasculum with moist cotton wool round the stems; an average of eight scions were taken at a time and only the freshest and most suitable were used, usually six. The lamp-glasses placed over the grafts were shaded by paper to ensure moist conditions.

Table I (*a*) gives particulars as regards those scions which developed mosaic disease, and Table I (*b*) as to those which remained healthy. As to the former, the symptoms of disease were the same as those obtained in the 1927 experiments. The upper leaves showed the characteristic mosaic mottling, curling and brittleness; the tip of the stem refused to climb and in some cases wilted and died back. In one case, OE 40 (ON 20), the scion reached the height of 274 cm., flowered and produced cones which showed distortion of bracts and bracteoles—one of the characteristic symptoms of mosaic disease. In addition, the leaves developed the symptoms.

Discussion of results.

The experiments carried out indicate that varieties of hops cultivated in Germany, America, Denmark and England are "carriers" of the mosaic disease. This fact, which became apparent during the work in 1927, was so surprising that before its acceptance the confirmation of another year's investigation was thought to be necessary. An explanation of the results obtained in 1927 might have been forthcoming if the plants used for scions in 1927 had shown mosaic disease in 1928, but this was not the case. We will discuss the results obtained class by class. The varieties of hops grafted fall into the following groups: (1) German; (2) American; (3) Danish; (4) English; (5) Seedlings; (6) Male Hops.

Group 1. *German varieties.*

(*a*) Aischgründ, Hallertau, Württemberg, Spalt, Prackenfels, Beck'scher Frühhopfen, Lower Bavarian.

(*b*) Tettnanger, Hübhopfen.

Healthy scions of susceptible varieties (Tutsham, Eastwell Golding, Cobbs) when grafted on the German varieties included above under (*a*) all developed typical mosaic disease (see Table I). It would appear therefore that all these German varieties growing at Wye are "carriers."

All the varieties under (*a*), with the exception of Lower Bavarian, were originally obtained in 1911 from Prof. Wagner of Weihenstephan, Germany, and were planted that year in the Experimental Hop Garden

at Wye. These varieties, comprising 25 plants, have during the period 1911–28 never shown any symptoms of mosaic, although this disease is very prevalent in the Experimental Hop Garden, where a considerable number of plants of other varieties in close proximity (in some cases actually adjoining) have, during the same period, been attacked and killed. The immunity shown is congruous with the now-ascertained fact of their being "carriers." Two other plants (clone-plants) of Hallertau used were obtained about 1912 from Dr Schmidt of Copenhagen under ref. no. Tysk B 149; the origin of these was likewise Weihenstephan.

The Lower Bavarian variety was planted at Wye at some date before 1906 and its origin is unknown, but is probably Germany. The five "hills" which have been growing at Wye since 1906 have never shown symptoms of mosaic disease.

The mosaic disease has not apparently been reported from Germany[1], but some German varieties are undoubtedly susceptible. Cases have occurred at Wye on the following: (1) plants obtained from some unknown source, prior to 1915, labelled "Upper Bavaria (Hallertau)"; the five plants were all destroyed by mosaic disease between 1917 and 1919; (2) plants obtained in 1916 from France (M. Blavier) of two varieties labelled "Alsace" and "Spalt." The entire stock, consisting of four plants of the former and five of the latter, has been destroyed by mosaic disease; (3) plants obtained in 1918 from Dr Quanjer of a variety labelled as a German variety and stated to have been obtained from Bavaria some 40 years previously and since cultivated at the Instituut voor Phytopathologie, Wageningen, Holland. Of this the stock of four plants has all been destroyed by mosaic disease during 1919–21.

No exact identification of the above four German varieties is possible; the fact, however, that they were wiped out by the mosaic disease while growing in close proximity to other German varieties, now shown to be "carriers," is of interest.

In view of the knowledge now gained that the Hallertau variety can serve as a "carrier," the following circumstances regarding an outbreak of mosaic disease seen some years ago on a certain farm in Kent, possibly acquire special significance. Between 1904 and 1908 a farmer obtained from Germany, through the medium of a London Hop merchant, cuttings of the Hallertau variety and grew them in a hop garden at Wateringbury, Kent. Some 200 sets were planted, with rows of the

[1] The disease called "mosaic Goldings" by Dr Blattny(1) does not agree, in the symptoms described, with mosaic disease as known in England.

mosaic-susceptible English variety Cobbs adjoining them on both sides. It was noticed in 1922, and for some years previously, that while the German variety remained healthy, many of the plants of Cobbs on both sides of them showed the mosaic disease, which was increasingly severe the nearer the plants were to the Hallertau variety. In 1922 the whole garden, including the Hallertau, was grubbed up and two years later was replanted with the Tutsham variety, since when no signs of mosaic disease have been noticed. The circumstantial evidence here would appear to be quite similar to that relative to the cases of the "carrier" seedlings M 45 and OD 8 (see pp. 378, 379).

In division (*b*), consisting of those stocks on which the susceptible scions remained healthy, are two varieties, Hübhopfen and Tettnanger. Both varieties were obtained in 1911 from Prof. Wagner; the first-named came from the Sortengarten des Hopfenbauzweigvereines Hersbrucker Land in Hersbruck, and the second from the Hopfenvarietätengarten, Weihenstephan. The five plants of these varieties have occupied a similar position during 1911–28 to that of the varieties now proved to be "carriers," and have never shown any signs of mosaic disease. The evidence that the variety Hübhopfen is not a "carrier" appears strong. In 1927, 17 scions were taken from one plant of the susceptible variety Tutsham (R 1/50 *a*); 16 of these were grafted on to the varieties Prackenfels, Beck'scher Frühhopfen, Golden Cluster, L 36, M 45, OE 40 and OQ 17 and all developed mosaic disease, while the remaining one grafted on the variety Hübhopfen remained healthy. Similarly, in 1928, two scions of Rodmersham Golding (ref. nos. 33 and 26) which were grafted on another individual of Hübhopfen remained healthy.

In the case of the Tettnanger variety, one scion of the susceptible variety Rodmersham Golding grew to 215 cm. and remained healthy; the second scion, taken from a wild hop (ref. no. 84), grew to 275 cm. and remained healthy. The evidence that this wild hop was susceptible (and would have shown mosaic disease were the Tettnanger variety a "carrier") is strong, since six other scions taken from it, when grafted on to the "carriers" Grön, OF 24, O 39 and OQ 17, in every case developed mosaic disease.

Further work is now required to confirm these results and, if confirmation is obtained, to investigate whether the varieties Hübhopfen and Tettnanger are susceptible to mosaic disease or are immune varieties without being "carriers."

One puzzling feature occurred in connection with the variety Hallertau. While it appears clear from the results recorded in Table I

that the variety is a "carrier," since of two plants (ref. nos. R 1/24, R 2/12) one transmitted mosaic disease to two scions of Eastwell Golding and the other to two scions of Tutsham, another plant of Hallertau behaved differently. This plant (ref. no. R 2/3) was grafted with a scion of the wild hop (ref. no. 86) which grew to the top wire and remained healthy. Now this wild hop was used also in another experiment when a scion put on the "carrier" M 45 (see Table I) developed mosaic disease. The plant ref. no. R 1/24 was obtained from Prof. Wagner at Weihenstephan, Germany, and the clone-plants ref. nos. R 2/3 and R 2/12, under the ref. no. "Tysk B 149," from Dr Schmidt, who informed us that the origin of these plants was Weihenstephan. Further investigations are required here.

Group 2. *American varieties.*

Californian Golden Cluster, Oregon Golden Cluster, Humphrey, Red Vine Canada.

Healthy scions of the susceptible varieties Tutsham, Rodmersham Golding, Cobbs, Eastwell Golding, and of a wild hop, when grafted on all the above varieties, developed mosaic disease. In one experiment eight scions were taken from a plant (ref. no. R 1/82 *a*) of Rodmersham Golding; four were grafted on the Californian Golden Cluster, and all developed mosaic; the same was the case with one scion grafted on the "carrier" Seedling L 40, whereas two scions grafted on the variety Golding Grape and one scion on the susceptible variety Bates Brewer remained healthy. It would appear clear therefore that all these four American varieties are "carriers." The Californian, and Oregon, Golden Cluster and Humphrey show a healthy growth and normal appearance in every way, but the Red Vine Canada sometimes shows in its leaves symptoms of a virus disease. These symptoms were first noticed in a clone-plant (ref. no. R 1/74) obtained in 1914 from Dr W. W. Stockberger of the U.S. Dept. of Agriculture under ref. no. 18, the original cuttings being from J. J. Bennett, Waterville, New York. In 1927 it was observed that the leaves, both young and old, showed distinct mosaic mottling although this was semi-obscured by the dark green colour of the lamina; no other sign of disease was apparent, the leaves were not curled or brittle, the tips of the bine and laterals were not affected, and the growth and fertility were not impaired. The mosaic mottling was so different from that found in ordinary mosaic disease that the term "masked mosaic" is applicable. This appearance is perhaps seasonal and did not occur in 1928. It may easily have been passed over

in previous years. The other clone-plant used of this variety (ref. no. R 1/70) has not shown any abnormal coloration of its leaves. Five clone-plants have been grown at Wye.

The origins of the other American hops, now proved to be "carriers," are as follows. The Golden Cluster (ref. no. R 1/58) was obtained from Dr Stockberger in 1914 under the ref. no. 7 C, the original cuttings being from near Cosumne, California. Five clone-plants have been grown at Wye. The Californian Golden Cluster (ref. nos. E 24 (clone-plants R 3/55, OZ 93) and OZ 92 (clone-plant R 3/57)) was obtained by Mr Arthur Amos, in 1911, from Mr E. C. Horst, Perkins, near Sacramento, California. Six plants have been grown at Wye. The Oregon Cluster (ref. no. C 8) was sent as an "Oregon Male" by Messrs Wigan, Richardson and Co.'s agent in Oregon in 1908. Two clone-plants have been grown at Wye. The Humphrey (ref. no. R 1/67) was sent by Dr Stockberger in 1914 under the ref. no. 44, the original cuttings being from H. V. Pindar, Middleburg, New York. Four clone-plants have been grown at Wye.

None of the plants of the above varieties has shown mosaic symptoms.

Whilst the above female American varieties have proved to be "carriers" and resistant, all the male hops obtained from the United States have proved to be extremely susceptible to the mosaic disease, so much so that it has been almost impossible to maintain a stock at Wye. Male hops have been obtained from the following sources. (1) A male plant (ref. no. R 1/51) sent by Dr Stockberger in 1914 under the name of Golden Cluster, ref. no. 7 A. Six clone-plants were attacked and killed by mosaic in 1920–23. (2) An Oregon male (ref. no. F 9), sent in 1908 by Messrs Wigan, Richardson and Co.'s agent in Oregon, succumbed to mosaic disease in 1914, and a clone-plant in 1924. Another Oregon male (ref. no. F 6) of same origin was killed by mosaic disease in 1912, and a clone-plant in 1915. Another Oregon male (ref. no. F 7) of same origin died from the same cause in 1914, and a clone-plant in 1917. Three others (ref. nos. G 35, F 14, F 17) were killed in 1910, 1909 and 1914 respectively. (3) Rooted sets of male hops from California were obtained in 1914 from Messrs Horst Co., the cuttings being originally from the Russian River, Mendocino County, California. Eight plants (ref. nos. OY 1–OY 8) were attacked and killed by mosaic between 1918 and 1922.

Group 3. *Danish variety.*

The Danish variety Grön was obtained from Dr J. Schmidt of Copenhagen about 1912, under the ref. nos. 199 and 198, the origin of

199 being given as Fyen, Denmark. Six clone-plants of 199 and four of 198 have been grown at Wye and have never shown mosaic symptoms. As will be seen from Table I, seven scions of Tutsham and of a wild hop were grafted on individuals of 199 and 198 and all developed mosaic disease. The variety Grön is therefore to be regarded as a "carrier."

Other Danish varieties sent by Dr Schmidt in 1911, it may be noted, are susceptible to mosaic disease. The variety Asperup (ref. no. 209) was attacked and killed by mosaic disease in 1915, and two clone-plants (ref. no. 210) perished of mosaic disease in 1915 and 1921. The variety Graa Rod from the Island of Fyen, Denmark, was killed by mosaic disease in 1921. A wild hop from Denmark (ref. no. Bork 291) proved to be very susceptible, nine clone-plants succumbing between 1919 and 1921.

Group 4. *English varieties.*

(*a*) Fuggles, Colgate, Allcorn, Golden Hop.

(*b*) Pye's Golding Grape.

The varieties in section (*a*) will be considered first. The Fuggles plant (ref. no. R 1/70 *a*) used in 1927 was of an English stock obtained from the late Mr G. Arnold. Two scions from a pot plant (ref. no. 47) of Tutsham were grafted on and both developed mosaic disease. One scion reached a height of 155 cm. and showed typical symptoms in the form of mottled, down-curled, and brittle leaves and, later, a non-climbing tip; the other scion grew to 73 cm. and, after showing mottled foliage, turned yellow and died.

In 1928 another plant (ref. no. R 1/68 *a*) of the same stock as above was grafted with Rodmersham Golding; the scion grew to 102 cm. and there were suspicious symptoms of disease, the leaves at many of the nodes showing a yellowish but not typical mosaic mottling, and the tip dying back in the manner characteristic of mosaic disease.

Other plants of the Fuggles variety were grafted in 1928. One plant (R 1/65) had been obtained in 1914 from Dr Stockberger, under ref. no. 9, from Sir A. Stephney's ranch, Agassiz, British Columbia, the original cuttings having come from East Kent, England. This plant was grafted with Rodmersham Golding; the scion grew to 48 cm. and a few of its leaves showed a faint but characteristic mosaic mottling.

Another plant (ref. no. R 4/97) is of an unknown (English) source, planted at Wye some time before 1917. The Tutsham scion grafted on reached to 43 cm. and its leaves showed faint mosaic mottling; the tip of the main stem and the laterals died back.

Table I.

Grafting experiments with susceptible scions on various varieties of hops.

(*a*) *Scions developing mosaic disease.*

1927 (D. MACKENZIE)

Stock		Scion		Suspected symptoms		Definite symptoms		Total height reached by scion (cm.)
Variety	Ref. no.	Variety	Ref. no.	Date	Height scion (cm.)	Date	Height scion (cm.)	
			1. *German varieties*					
Aischgründ	R 1/12	Tutsham	38	5. vii.	23	3. viii.	23	23
,,	R 1/12	Eastw. Gold.	76	—	—	9. vi.	61	62
,,	R 1/20	,,	72	3. viii.	278	30. viii.	325	325
,,	R 1/20	,,	78	9. viii.	295	30. viii.	317	317
Hallertau	R 1/24	,,	63	5. vii.	33	12. vii.	33	33
,,	R 1/24	,,	62	22. vi.	35	29. vi.	40	41
,,	R 2/12	Tutsham	1	19. vii.	80	23. viii.	128	128
,,	R 2/12	,,	1	23. viii.	195	5. ix.	195	195
Württemberg	R 1/32	Eastw. Gold.	71	22. vi.	110	3. viii.	200	215
,,	R 1/32	,,	66	—	—	3. viii.	310	310
Spalt	R 1/36	Cobbs	R 1/36 *a*	23. viii.	270	30. viii.	270	270
,,	R 1/36	,,	R 1/36 *a*	16. viii.	260	5. ix.	265	265
Prackenfels	R 1/39	Tutsham	R 1/50 *a*	—	—	16. vi.	85	100
,,	R 1/39	,,	R 1/50 *a*	—	—	16. vi.	85	123
Lower Bavarian	R 3/15	,,	7	16. vi.	85	19. vii.	155	212
,, ,,	R 3/15	,,	7	—	—	16. vi.	70	165
Beck'scher Frühhopfen	R 1/45	,,	R 1/50 *a*	12. vii.	198	5. ix.	250	250
,, ,,	R 1/45	,,	R 1/50 *a*	3. viii.	220	5. ix.	260	260
			2. *American varieties*					
Golden Cluster	R 1/58	Wild Hop	83	12. vii.	46	9. viii.	30*	46
Red Vine Canada	R 1/70	Tutsham	31	26. vii.	31	9. viii.	31	31
,, ,,	R 1/74	,,	46	—	—	16. vi.	72	195
Cal. Golden Cluster	E 24	,,	R 1/50 *a*	22. vi.	35	29. vi.	38	112
,, ,,	E 24	,,	R 1/50 *a*	16. vi.	47	22. vi.	47	47
,, ,,	R 3/55	,,	R 1/50 *a*	12. vii.	187	19. vii.	190	210
,, ,,	R 3/55	,,	R 1/50 *a*	12. vii.	112	19. vii.	120	150
,, ,,	OZ 93	Rodm. Gold.	R 1/82 *a*	5. vii.	141	12. vii.	155	335
,, ,,	OZ 93	,,	R 1/82 *a*	16. vi.	51	22. vii.	60	143
,, ,,	OZ 92	,,	R 1/82 *a*	—	—	16. vi.	40	46
,, ,,	OZ 92	,,	R 1/82 *a*	16. vi.	75	22. vi.	81	115
,, ,,	R 3/57	Tutsham	R 1/50 *a*	—	—	16. vi.	45	49
Oregon Cluster	C 8	Cobbs	R 1/36 *a*	22. vi.	25	29. vi.	25	25
			3. *Danish variety*					
Grön 199	R 2/2	Wild Hop	84	19. vii.	70	26. vii.	70	70
,,	R 2/11	Tutsham	13	9. viii.	160	5. ix.	187	187
,,	R 2/11	,,	13	3. viii.	230	16. viii.	255	255
Grön 198	R 2/7	Wild Hop	85	26. vii.	195	16. viii.	260	265
,,	R 2/7	,,	85	26. vii.	95	9. viii.	125	135
,,	R 2/16	Tutsham	17	9. viii.	155	16. viii.	158	158
,,	R 2/16	,,	17	9. viii.	290	16. viii.	310	315

* Died back.

Table I (*continued*).

1927 (D. Mackenzie) (*continued*)				Suspected symptoms		Definite symptoms		Total height reached by scion (cm.)
Stock		Scion			Height scion (cm.)		Height scion (cm.)	
Variety	Ref. no.	Variety	Ref. no.	Date		Date		
			4. English varieties					
Fuggles	R 1/70 *a*	Tutsham	47	22. vi.	90	29. vi.	95	155
,,	R 1/70 *a*	,,	47	—	—	16. vi.	56	73
Colgate	R 1/84	Rodm. Gold.	81 *a*	5. vii.	146	19. vii.	147	147
,,	R 1/84	,,	81 *a*	12. vii.	221	3. viii.	190*	221
,,	R 1/85	,,	81 *a*	12. vii.	164	26. vii.	165	165
,,	R 1/85	,,	81 *a*	12. vii.	165	19. vii.	165	165
Golden Hop	341	Wild Hop	81	29. vi.	33	12. vii.	33	33
,,	288	Eastw. Gold.	63	—	—	16. vi.	51	65
,,	(Bide's)	,,	27	5. vii.	45	3. viii.	30*	45
,,	(Bide's)	Tutsham	29	12. vii.	43	19. vii.	45	45
			5. New Seedling varieties					
S 20	R 2/63	Tutsham	26	5. vii.	78	12. vii.	80	81
,,	R 2/63	,,	26	22. vi.	120	29. vi.	120	120
L 21	L 21	,,	41	—	—	3. viii.	44	44
,,	L 21	,,	41	—	—	3. viii.	127	127
,,	OZ 61	,,	47	22. vi.	45	12. vii.	50	50
,,	OZ 60	,,	3	9. viii.	63	16. viii.	75	75
OP 21	R 4/49	,,	31	—	—	16. vi.	60	130
,,	R 4/49	,,	31	22. vi.	88	29. vi.	91	122
,,	OK 17	,,	33	23. vi.	30	29. vi.	30	30
,,	OK 17	,,	34	23. vi.	59	29. vi.	60	61
,,	R 4/43	,,	8	22. vi.	58	29. vi.	60	85
,,	R 4/43	,,	8	16. vi.	54	22. vi.	60	125
,,	R 4/50	Wild Hop	81	29. vi.	162	3. viii.	250	280
,,	R 4/50	,,	81	22. vi.	72	3. viii.	120	130
OF 30	OF 30	Eastw. Gold.	44	—	—	16. vi.	40	52
,,	OF 30	,,	44	16. vi.	47	23. vi.	49	49
,,	R 2/49	Tutsham	13	—	—	16. vi.	52	53
M 54	OZ 17	,,	22	—	—	3. viii.	210	Top†
,,	OZ 17	,,	22	26. vii.	210	3. viii.	Top	Top
,,	221	,,	36	—	—	16. vi.	50	157
,,	221	,,	36	22. vi.	81	29. vi.	92	208
,,	222	Eastw. Gold.	68	16. vi.	70	22. vi.	78	185
,,	222	,,	62	16. vi.	60	22. vi.	66	147
,,	158	,,	73	16. vi.	80	22. vi.	99	260
,,	158	,,	79	22. vi.	90	29. vi.	107	280
L 36	L 36	Tutsham	15	26. vii.	65	3. viii.	65	65
,,	L 36	,,	16	19. vii.	74	3. viii.	80	80
,,	J 51	,,	R 1/50 *a*	5. vii.	107	19. vii.	125	150
,,	J 51	,,	R 1/50 *a*	5. vii.	109	19. vii.	145	185
L 40	40	Rodm. Gold.	R 1/83 *a*	—	—	3. viii.	78	78
,,	40	,,	R 1/82 *a*	—	—	16. vi.	63	64
M 45	153	Eastw. Gold.	72	29. vi.	131	12. vii.	160	160
,,	G 15	Wild Hop	85	22. vi.	95	12. vii.	120	155
,,	G 15	,,	86	22. vi.	97	29. vi.	107	177
,,	G 16	Tutsham	R 1/50 *a*	12. vii.	93	19. vii.	101	122
,,	G 16	,,	R 1/50 *a*	29. vi.	88	12. vii.	108	128
OF 24	11	,,	40	22. vi.	58	29. vi.	61	67
,,	V 42	,,	4	22. vi.	72	29. vi.	72	72
,,	V 42	,,	6	22. vi.	94	29. vi.	97	98
,,	OF 24	Wild Hop	84	16. vi.	49	23. vi.	50	50
,,	OF 24	,,	84	23. vi.	34	29. vi.	34	34
O 39	105	,,	81	22. vi.	52	29. vi.	56	58
,,	105	,,	84	22. vi.	81	29. vi.	88	89
,,	DD 30	Tutsham	42	12. vii.	43	26. vii.	45	45
,,	DD 30	,,	37	29. vi.	40	5. vii.	41	41

* Died back.

† The height of the wirework varies from 12 to 14 feet, *i.e.* from 3·7 to 4·3 metres, from the ground.

Table I (*continued*).

1927 (D. MACKENZIE) (*continued*)

Stock		Scion		Suspected symptoms		Definite symptoms		Total height reached by scion (cm.)
Variety	Ref. no.	Variety	Ref. no.	Date	Height scion (cm.)	Date	Height scion (cm.)	
		5. *New Seedling varieties* (*continued*)						
P 13	D 18	Cobbs	R 1/36 *a*	—	—	16. vi.	20	20
,,	D 18	,,	R 1/36 *a*	16. vi.	53	22. vi.	54	56
,,	D 8	Tutsham	19	22. vi.	49	29. vi.	51	51
,,	D 8	,,	19	16. vi.	86	22. vi.	100	200
OH 8	OH 8	,,	9	23. vi.	53	12. vii.	75	110
,,	OH 8	,,	32	—	—	3. viii.	25	25
B 7	B 7	,,	19	3. viii.	125	30. viii.	140	140
,,	B 7	,,	0	12. vii.	102	19. vii.	120	175
,,	E 7	Cobbs	R 1/36 *a*	9. viii.	50	16. viii.	55	55
OE 40	OE 40	Tutsham	29	29. vi.	102	5. vii.	112	147
,,	X 38	,,	1	29. vi.	29	26. vii.	29	29
,,	X 38	,,	1	23. vi.	109	29. vii.	114	135
,,	ON 20	,,	R 1/50 *a*	29. vi.	74	12. vii.	74	74
Y 86	Y 86	Cobbs	R 1/36 *a*	23. vi.	65	29. vi.	71	150
,,	Y 86	,,	R 1/36 *a*	—	—	16. vi.	71	160
,,	Y 83	,,	R 1/36 *a*	—	—	16. vi.	30	30
,,	Y 83	,,	R 1/36 *a*	—	—	16. vi.	33	35
OD 8	OD 8	Eastw. Gold.	70	29. vi.	32	26. vii.	40	40
,,	OD 8	,,	65	23. vi.	45	29. vi.	46	47
,,	OG 59	Tutsham	26	—	—	9. viii.	25	25
,,	EE 6	,,	17	12. vii.	66	19. vii.	70	90
O 52	OD 29	,,	42	5. vii.	57	19. vii.	77	100
,,	OD 29	,,	42	5. vii.	125	12. vii.	150	260
,,	OD 56	,,	1	29. vi.	91	12. vii.	128	210
,,	OD 56	,,	1	23. vi.	120	5. vii.	155	260
OQ 17	OQ 17	,,	R 1/50 *a*	—	—	3. viii.	98	100
,,	OQ 17	,,	R 1/50 *a*	—	—	3. viii.	88	88
,,	OF 31	Eastw. Gold.	44	29. vi.	123	19. vii.	165	185
,,	OF 35	,,	52	5. vii.	62	26. vii.	83	92
,,	OF 35	,,	48	29. vi.	60	5. vii.	68	105
,,	OF 36	Wild Hop	84	16. vi.	113	23. vi.	129	150
,,	OF 36	,,	84	23. vi.	120	29. vi.	131	200
OG 1	OG 1	Tutsham	56	23. vi.	29	29. vi.	29	30
310	OJ 22	,,	20	29. vi.	27	3. viii.	27	27
,,	OJ 22	,,	20	12. vii.	56	19. vii.	56	56
,,	OJ 12	Wild Hop	85	5. vii.	56	12. vii.	61	90
L 7	CC 1	Tutsham	31	5. vii.	108	12. vii.	108	108
,,	CC 2	,,	5	29. vi.	51	19. vii.	52	52
		6. *Male Hops*						
OI 22	OI 22	Tutsham	5	29. vi.	54	12. vii.	54	54
,,	OI 22	,,	5	—	—	12. vii.	60	70
G 27	II 2	,,	43	16. vi.	79	23. vi.	81	81
,,	II 2	Wild Hop	85	5. vii.	60	12. vii.	60	60
,,	II 3	Tutsham	43	16. vi.	28	29. vi.	30	30
,,	II 4	,,	9	—	—	30. viii.	325	325
,,	II 4	,,	43	16. vi.	99	29. vi.	112	114
,,	II 5	,,	16	12. vii.	62	19. vii.	75	125
,,	II 5	Eastw. Gold.	51	3. viii.	250	24. viii.	300	300
,,	II 6	Tutsham	45	5. vii.	60	12. vii.	65	70
,,	II 6	,,	25	—	—	26. vii.	50	58
,,	II 8	,,	45	—	—	9. viii.	210	230
,,	II 12	,,	25	—	—	3. viii.	60	60
B 11	T 36	,,	5	12. vii.	78	26. vii.	82	82
,,	T 36	,,	6	29. vi.	43	12. vii.	55	56
,,	T 43	,,	9	22. vi.	79	29. vi.	81	84
,,	T 54	,,	30	5. vii.	30	3. viii.	30	30
,,	T 66	,,	30	19. vii.	36	3. viii.	36	36

Table I (*continued*).

1928 (R. Williams)

Stock		Scion		Total height of scion (cm.)	Symptoms
Variety	Ref. no.	Variety	Ref. no.		
		1. *German varieties*			
Spalt	R 1/36	Eastw. Gold.	R 15/H 14	210	p.M.
Prackenfels	R 1/40	,,	R 16/H 5	89	? M.
		2. *American variety*			
Humphrey	R 1/67	Eastw. Gold.	R 1/H 7	74	M.
		3. *English varieties*			
Fuggles (B.C.)	R 1/65	Rodm. Gold.	28	48	? M.
Fuggles	R 1/68 *a*	,,	28	102	? M.
,,	R 4/97	Tutsham	21	43	? M.
,,	R 2/10	,,	R 1/19 *a*	79	? M.
Golden Hop	288	Eastw. Gold.	R 3/H 5	117	? M.
Allcorn	OP 97	,,	R 12/H 3	69	M.
		4. *New Seedling varieties*			
S 20	S 20	Eastw. Gold.	R 1/H 17	59	? M.
L 21	OZ 59	,,	R 1/H 22	74	M.
,,	OZ 60	,,	R 1/H 25	53	M.
OP 21	S 55	,,	R 2/87	216	M.
,,	S 60	,,	R 2/H 8	206	M.
L 40	40	,,	R 2/H 17	71	M.
458	457	,,	R 3/H 2	125	M.
,,	458	,,	R 3/H 2	152	M.
OF 24	OF 24	,,	—	125	M.
,,	V 42	,,	R 3/H 9	114	M.
B 7	E 7	,,	R 4/H 3	175	M.
,,	E 7	,,	R 4/H 3	196	M.
OE 40	OE 40	,,	R 4/H 5	109	M.
,,	OE 40	,,	R 4/H 5	69	M.
,,	X 38	,,	R 4/H 9	173	M.
,,	X 38	,,	R 4/H 6	64	M.
,,	ON 20	,,	R 4/H 7	274	M.
OD 8	EE 6	,,	R 10/H 1	82	p.M.
,,	OG 59	,,	R 8/H 0	155	? M.
,,	OG 59	,,	R 8/H 1	41	? M.
,,	OX 26	,,	R 8/H 9	26	? M.
OQ 17	OF 35	,,	R 10/H 14	152	M.
L 7	CC 2	,,	R 12/H 1	114	? M.
		5. *Male Hops*			
R 2/20	R 2/20	Eastw. Gold.	R 22/H 8	318	? M.
R 2/7 *a*	R 2/7 *a*	,,	R 22/H 8	61	? M.

M. = Mosaic; p.M. = probable Mosaic.

Table I (*continued*).

(*b*) *Scions remaining healthy.*

Stock		Scion		Total height of scion (cm.)
Variety	Ref. no.	Variety	Ref. no.	
1927 (D. MACKENZIE)				
		1. *German varieties*		
Tettnanger	R 1/15	Wild Hop	84	275
,,	R 1/15	Eastw. Gold.	61	215
Hallertau	R 2/3	Wild Hop	86	Top
Hübhopfen	R 1/42	Tutsham	R 1/50 *a*	—
		2. *English varieties*		
Pye's Gold. Grape	R 1/89	Tutsham	21	125
,, ,,	R 1/91	,,	10	283
,, ,,	R 1/90	Rodm. Gold.	R 1/82 *a*	195
,, ,,	R 1/90	,,	R 1/82 *a*	300
,, ,,	R 1/91	Eastw. Gold.	67	265
Bates Brewer	R 2/90	Rodm. Gold.	R 1/82 *a*	Top
,,	R 2/90	,,	R 1/83 *a*	Top
,,	R 2/88	,,	R 1/83 *a*	Top
		3. *New Seedling varieties*		
OQ 17	OF 31	Rodm. Gold.	44	Top
		4. *Male Hop*		
B 11	T 66	Tutsham	30	122
1928 (R. WILLIAMS)				
		1. *German variety*		
Hübhopfen	R 1/43	Rodm. Gold.	33	259
,,	R 1/43	,,	26	256
		2. *English variety*		
Fuggles	R 4/99	Tutsham	R 1/23	64

The fourth plant (ref. no. R 2/10) had been obtained in 1911 from Dr Schmidt, under the ref. no. Eng. B 175, the original cuttings having come from Wye. The Tutsham scion grafted on grew to 79 cm. and its leaves showed very little and faint mosaic mottlings.

All the above experiments in 1928 must be regarded provisionally as doubtful cases of the transmission of mosaic disease[1].

A number of plants of the Fuggles variety, growing in large pots, were grafted in 1928 by W. M. Ware. These plants were obtained from Mr Ingleby, Hereford, and were of three different stocks, *A*, *B* and *C*. The results are shown in Table II. The scions used were Eastwell

[1] One plant (ref. no. 4/99) of Fuggles, of an unknown English source, planted at Wye sometime before 1917, was grafted with a Tutsham scion; this grew to 64 cm. and remained healthy.

Table II.

Susceptible scions (Eastwell Golding and Tutsham) grafted on the variety Fuggles.

Order in which grafting carried out	Ref. no. of stock (variety Fuggles)	Ref. no. and variety* of scion	Date grafted	Height of scion on June 1st (cm.)	Scion		
					Healthy	Weak growth possibly due to virus	Undoubted mosaic symptoms
23	B 1	E.G. W 46	27. iii.	30	—	×	—
11		T. 5	19. iii.	38	—	—	×
13	B 2	E.G. W 26	19. iii.	12·5	—	×	—
14		T. 24	19. iii.	79	×	—	—
16	A 1	E.G. W 32	27. iii.	37·5	—	—	×
15		T. 45	27. iii.	66·5	—	—	×
17	A 2	E.G. 70	27. iii.	165	×	—	—
18		T. 9	27. iii.	188	×	—	—
22	B 3	T. 2	27. iii.	27	—	—	×
26	B 4	E.G. 70	2. iv.	14	—	×	—
27		T. 22	2. iv.	24	—	—	×
1	C 1	E.G. W 2	17. iii.	39	—	×	—
21		T. 30	27. iii.	116	×	—	—
10	C 2	E.G. W 20	19. iii.	16	—	×	—
2		T. 25	17. iii.	25	—	×	—
3	C 3	E.G. 76	17. iii.	17	—	×	—
25		T. 13	2. iv.	99	—	×	—
24	C 4	E.G. W 48	2. iv.	43	—	×	—
4		T. 0	17. iii.	111	×	—	—
5	B 5	E.G. 65	17. iii.	17	—	—	×
12		T. 22	19. iii.	71	—	—	×
6	A 3	E.G. 79	17. iii.	176	×	—	—
20		T. 23	27. iii.	62	—	—	×
7	A 4	E.G. 75	17. iii.	12	—	×	—
19		T. 12	27. iii.	135	×	—	—
8	A 5	E.G. W 16	19. iii.	17	—	×	—
9		T. W 18	19. iii.	18	—	×	—

* E.G. = Eastwell Golding; T. = Tutsham.

Golding (E.G.) and Tutsham (T.). It will be seen that of the 27 scions grafted, 8, or 29·6 per cent., developed characteristic symptoms of mosaic disease. These were evident in the typical mottling of six of the 18 to 26 leaves on four of the scions and of eight out of 18 to 26 leaves on the other four.

Twelve scions, or 44·4 per cent., grew weakly and seven scions, or 25·9 per cent., remained perfectly healthy and reached heights of from 79 to 188 cm. The scions and stocks, when the grafting was carried out, were well matched and the operation was performed under the best conditions. Normally with such vigorous plants as stocks, the scions are expected to grow rapidly and to attain easily the height of 188 cm.

which in this experiment resulted only with the healthiest on one stock plant. All the Fuggles were of equal vigour and the poor growth of the twelve scions may perhaps be attributed to the same cause, *i.e.* virus attack, as in the eight which showed evident symptoms.

The stocks of Fuggles used were not clone-plants, but were obtained from a hop grower. Admixture is notoriously common in commercial stocks of varieties of hops, and the possibility must not be lost sight of that the plant A 2, on which both scions remained healthy and grew vigorously, may be of another variety, or a male hop. If this explanation may hold good for this case, we are still confronted with a difficulty as regards the plant A 3, where one scion (Eastwell Golding) remained healthy and grew to 176 cm., while the other (Tutsham) grew to only 62 cm. and developed mosaic disease. This case is somewhat similar to that of OQ 17 discussed on p. 376. The fact also may be noted that in the case of plant C 1, the Tutsham scion which remained healthy was taken from the same plant (ref. no. 30) as the one scion which remained healthy, in 1927, when grafted on the male hop B 11; two other scions from this same Tutsham plant developed mosaic disease when grafted, in 1927, on the same plant of B 11 and on another clone-plant.

Whilst it is clear that further investigations are here necessary, the positive results obtained in the three sets of experiments carried out in 1927 and 1928 appear to give safe grounds for stating that the Fuggles variety is, to some extent at least, a "carrier."

The Colgate plants grafted (ref. nos. R 1/84, R 1/85) were obtained from Mr A. W. Wallace, Battle, Sussex, in 1914. From that date to 1928, the four plants of this stock growing at Wye have never shown mosaic disease. Four scions of Rodmersham Golding were grafted on and all developed mosaic disease. It appears, therefore, that this variety is a "carrier."

In 1928 one plant of the variety Allcorn was grafted with one scion of Eastwell Golding. This scion, which grew to 69 cm., showed typical mosaic mottling on its leaves. Although the evidence here is slight, it would appear that the Allcorn variety, the origin of which is unknown, may be a "carrier." The variety was obtained in 1925 from the late Mr H. C. Wickham of Ticehurst.

The Golden Hop is a variety with yellow (golden) leaves. It is in use for horticultural purposes only. Its origin, so far as it can be traced, has already been dealt with (4). All of the five scions grafted (Eastwell Golding, Tutsham, and a wild hop) developed mosaic disease.

Section (*b*). The variety Pye's Golding Grape was obtained in 1914

from Mr William Pye, of Cuxton, Kent. No details of its origin are obtainable. Five scions of the varieties Tutsham, Eastwell Golding and Rodmersham Golding were grafted on three individuals (ref. nos. R 1/89–91) and all remained perfectly healthy. In the case of R 1/90 the evidence that the variety is not a "carrier" is particularly strong. Eight scions were taken from a plant of Rodmersham Golding (R 1/82 *a*); four were put on the California Golden Cluster, one on the Seedling L 40, two on the Golding Grape (ref. no. R 1/90) and one on Bates Brewer. The first two varieties named proved to be "carriers" and transmitted mosaic to the five scions; those on the Golding Grape and Bates Brewer remained healthy. The ten plants of Pye's Golding Grape grown at Wye since 1914 have never been attacked by mosaic disease. Investigations will now be carried out to ascertain whether this variety is susceptible or is truly immune without being a "carrier."

The variety Bates Brewer is known to be susceptible to the mosaic disease and it is therefore not surprising that it does not behave as a "carrier." The fact that the scions grafted on it remained healthy was useful evidence of the health of the parent plants.

Group 5. *Seedling varieties.*

All the Seedling varieties enumerated in Table I, with the exception of B 7, of which the origin is unknown, are Seedlings raised at Wye College; they have subsequently been sent as promising varieties to the Research Station, East Malling, for trial on commercial lines. The parentage of each and their cropping and other characteristics have been given in Annual Reports(5). The evidence both in 1927 and 1928 is conclusive that these Seedlings are "carriers." In many cases grafts were made on several clone-plants of the same Seedling; thus OE 40 was grafted with ten scions (of Tutsham and Eastwell Golding) and all developed mosaic disease; the same was the case with the nine scions grafted on OP 21. Scions taken from an individual plant of Tutsham remained healthy when grafted on the German variety Hübhopfen, but contracted mosaic disease when grafted on the Seedlings L 36, M 45, OE 40 and OQ 17. Similarly, scions taken from the wild hop (No. 84) remained healthy when grafted on the German variety Tettnanger, and developed mosaic disease on the Seedlings OF 24, O 39 and OQ 17. Scions taken from one plant of Rodmersham Golding remained healthy on the English variety Bates Brewer, and showed mosaic disease when grafted on the Seedling L 40; from another plant of Rodmersham Golding, the scions remained healthy on the variety Golding Grape and developed mosaic

disease on the Seedling L 40. Corroboration of the results obtained in 1927 was obtained in 1928; using a new stock of scions obtained from a commercial hop garden of the variety Eastwell Golding, it was found that mosaic disease was transmitted to every scion grafted on the Seedlings L 21, OP 21, L 40, OF 24, OE 40, OQ 17. In addition the Seedling 458 was found to be a "carrier." The symptoms shown were quite typical of the disease, the leaves of the stem, and also frequently of the lateral shoots, showing the characteristic mosaic mottling and becoming very brittle; the tips of the shoots ceased to climb, and in some cases died back. All these cases are classified as "Mosaic" in Table I, and those as "? Mosaic" in which the symptoms, though suggestive of the disease, in that the leaves showed traces of mottling and that sometimes the tip of the stem died back, were not marked enough to make the diagnosis certain. The rather weak growth made by many scions under the seasonal conditions was unfavourable for the manifestation of the disease.

The fact that every Seedling variety tested proved to be a "carrier" is at first sight certainly very surprising. The question naturally suggests itself as to whether there can be any correlation with parentage. Detailed information as to the parentage of each Seedling will be found in the Annual Reports (5), but here a general summary may be given. Of the 22 Seedlings, 12 are of German and 4 of American parentage, while one was raised from the Golden Hop. In these cases the mother plant is either known to be or may be suspected of being a "carrier." Two Seedlings were raised from the Tolhurst variety, two from Canterbury Whitebine, and one from the Danish variety, Asperup—these three parents being all susceptible to mosaic disease. The male hop may here have had an influence.

The variety B 7 is of unknown origin and may possibly be a Seedling. It is of interest in being immune though showing the characters of certain English Golding varieties, all of which are susceptible to mosaic disease; it is now proved to be a "carrier."

It must be pointed out that only those Seedlings raised in the Nursery at Wye College which have shown over a number of seasons no signs of mosaic disease are sent to the Research Station at East Malling for commercial trial. All the Seedlings noted above have been thus selected and it is therefore not so surprising as it at first appears that all the twenty-two Seedlings tested have proved to be "carriers," since no certain case is known at present among hops of a variety being resistant to mosaic disease and not carrying the virus. Whilst being grown at

East Malling, none of the Seedlings has shown any trace of the disease, although commercial susceptible varieties growing adjacent have annually suffered severely (see Annual Reports (5)).

One of the most interesting anomalies was met with in 1927. Three clone-plants of the Seedling OQ 17 were grafted with six scions and all the scions developed mosaic disease. In 1928 one of these plants was again grafted and transmitted mosaic disease. It is clear therefore that OQ 17 is a "carrier." In 1927 a fourth clone-plant (ref. no. OF 3) of OQ 17 was grafted with two scions of Eastwell Golding taken from the same pot plant (ref. no. 44[1]); one developed mosaic disease while the other remained perfectly healthy, grew to over the top wire, flowered and produced cones. Certain other similar cases have been met with, viz. with Fuggles (see p. 373) and with the Male Hop ref. no. B 11 (see below).

Such cases can be explained hypothetically by assuming either (1) that the stock plant, although a "carrier" as regards certain shoots coming from the rootstock, produces other shoots which carry no virus or an insufficient amount; or (2) that the plant from which scions are taken may produce a bud-sport which is resistant to mosaic disease, whether carrying the virus or not.

Group 6. *Male Hops.*

Conclusive proof was obtained in 1927 that a male hop (ref. no. G 27) growing in the Experimental Hop Garden at Wye, and raised by A. Howard in 1904 from the Colgate variety, is a "carrier." Eleven scions of the varieties Tutsham and Eastwell Golding and of a wild hop all developed mosaic disease when grafted on seven clone-plants of G 27. With regard to another male plant (ref. no. B 11) of unknown origin, four scions of Tutsham grafted on three clone-plants (T 36, T 43, T 54) developed mosaic disease; on another clone-plant (T 66) two scions from the same plant (ref. no. 30) of Tutsham were grafted; one scion developed mosaic disease while the other grew to 122 cm. and remained healthy. The male hop B 11 is clearly a "carrier," and further investigations are necessary to explain why the one scion remained healthy. Clone-plants of both G 27 and B 11 have been grown at Wye and at the Research Station, East Malling, for several years past, and although almost certainly exposed to infection, have shown no trace of mosaic disease.

The male hop OI 22 (of the parentage Golden Hop × English Male

[1] Two scions of this plant grafted on the Seedling OF 30 both developed mosaic disease.

Hop × English Male Hop) was grafted with two scions of Tutsham and both developed mosaic disease (see Table I).

The existence of "carriers" among male hops[1] is a fact of considerable economic importance. Both G 27 and B 11 are of robust growth, of perfectly healthy appearance, have normal green foliage and are free-flowering, and on account of these characters have been propagated. Male hops are necessary in England for the production of a satisfactory crop, and are regularly planted by many farmers in the proportion of one male plant to 100–200 female plants. It is obvious that the planting of any "carrier" male hops will endanger the health of gardens which consist of commercial varieties susceptible to mosaic disease.

General considerations.

The discovery of the general prevalence in varieties of hops of the power of "carrying" the virus of mosaic disease without showing it raises many problems. One may speculate whether it will be found that the German, American and Danish varieties enumerated in Table I are "carriers" when growing in their own countries, or whether possibly they became "carriers" by infection abroad. With respect to the Seedlings enumerated, the indications are that these were "carriers" from the seed stage.

In the case of the English varieties, Fuggles and Colgate, the fact that these are grown in our country on heavy soils only, while the mosaic-susceptible Golding varieties require lighter soils, makes it unlikely that, in general, hop gardens of the latter varieties will be exposed to infection from the sources in question. On the Continent, where the Fuggles is now beginning to be cultivated on account of the resistance of its cones to the Downy Mildew, mosaic disease may well appear surreptitiously—assuming that all stocks of Fuggles are "carriers."

The discovery of "carriers" among male hops makes it desirable that further investigations should be made to ascertain whether the male hops (which are of no definite variety) planted in commercial hop gardens belong to the class of "carriers."

In the case of the New Seedling varieties, some of which have recently been used in brewing trials (8, 9) with promising results, it is obviously unsafe to plant such "carriers" in the proximity of susceptible Golding varieties; they must be grown either in separate gardens or near the

[1] In 1928 two male Seedling hops (ref. nos. R 2/20, R 2/7 *a*), both raised from the Golden Hop, were each grafted with one scion of Eastwell Golding. The leaves of both scions showed faint mosaic mottlings. These must be classed at present among the doubtful cases.

resistant ("carrier") commercial varieties Fuggles or Colgate. The question as to what distance should intervene between susceptible varieties and "carriers" can only be answered when it is known how mosaic disease is spread. At present nothing is known on this subject. Several ways in which the virus may be transmitted can be hypothecated. The Aphis, *Phorodon humuli*, and other insect parasites of the Hop may be agents; another possible animal vector may be the Eelworm, *Heterodera schachtii*, which Percival (3) and Duffield (2) have shown to infect the roots of the hop plant. The fine rootlets which develop in mass in the rich soil of the hop garden are torn apart and dispersed to some extent by the implements used in cultivating deeply, and fragments carrying eelworms may very possibly reach new uninfected gardens. The manual operation carried out each season of stripping off the leaves from the bines (stems) of the hop plants may play an important part in the spread of mosaic disease; the workers' hands, moistened with the sap of the leaves and shoots pulled off, may carry the virus and communicate it to adjacent varieties when the bines of these are in their turn wounded in the process of being stripped. In a similar manner it appears not improbable that in the annual operation of cutting the rootstock of the plant, the virus-infected sap may be transmitted by the knife from "carrier" varieties[1]. At the Research Station, East Malling, where all of the New Seedling Varieties mentioned above are being grown (together with "carrier" varieties such as Grön, Hallertau, Colgate and Fuggles) the present condition of affairs is such that as a row of plants of any susceptible Golding variety is planted in the proximity of the Seedling and other varieties, the row becomes almost at once decimated, and sometimes exterminated, by mosaic disease. Now that so many Seedling varieties have been proved to be "carriers," this rapid spread of mosaic disease could be explained on the assumption that in cultural operations, perhaps stripping, the Golding varieties become quickly infected.

A case where the Hallertau variety acted apparently as the "carrier," is noted on p. 362. Two further cases of the sudden appearance of the disease—inexplicable at the time but now probably to be explained by the agency of "carrier" varieties—may be mentioned. About ten years ago sets of the New Variety M 45 were distributed to two farmers at Wateringbury and Tunstall, Kent; they were planted for trial in gardens of mosaic-susceptible commercial varieties (Tutsham, Cobbs and Bramling),

[1] The possibility of soil infection in the case of certain mosaic diseases is indicated by the recent work of H. H. McKinney and R. W. Webb (*Journ. Agric. Research*, xxxv, 13 (1927) and xxxvi, 53 (1928)).

forming rows which were immediately surrounded by those of the susceptible variety. Under these conditions a severe outbreak of the disease occurred within three years of the planting[1]. Recently another New Variety, OD 8, has been tried on the same farm at Wateringbury and again the mosaic disease appeared in the adjoining rows in the hop garden. Our present knowledge that both M 45 and OD 8 are "carriers" provides us with the probable explanation in these cases, although we are still ignorant of the means whereby in nature the virus is transported from "carrier" plants. The transmission of mosaic disease has been demonstrated only by grafting, but it is abundantly clear that the disease spreads by some other means in hop gardens.

It may be pointed out here that the mosaic disease of the hop is comparatively new to this country. It was first described in 1923, but records kept at Wye show that the first occurrences noted there were during the years 1907 to 1910. It is idle, in our present ignorance, to speculate as to the origin of the disease, whether it is specific to the hop, or whether it may possibly be communicated from some other plant. If German and American varieties have always been "carriers," this source has been present in Kent for many years past. A hop garden of German varieties existed at Wye College some time before 1900. In other parts of Kent, and also in Sussex, the German variety Hallertau has been grown on an experimental scale by a few hop growers; American varieties (Palmer's Seedling and Oregon Cluster) were planted experimentally by hop growers at Horsmonden and Teynham, Kent, many years before 1900.

II. Resistance of the Fuggles variety when grafted on a "carrier."

In the experiments made by D. Mackenzie in 1927 and tabulated below, scions of the variety Fuggles were grafted on varieties now known to be "carriers."

In every case the scion grew well and remained healthy[2].

[1] Further details will be found in the Sixth Annual Report(5).

[2] In other experiments carried out by D. Mackenzie in 1927, and by R. Williams in 1928, the variety Fuggles was grafted with scions taken from another individual of the same variety (not clone-plants). Eleven experiments of this nature were carried out. All the scions grew well, in many cases reaching the top wire, remained healthy, and produced cones. In two experiments the variety Fuggles (ref. nos. R 4/99, R 1/61 *a*) was self-grafted with the same result. In 1927, two scions of Fuggles were grafted on the German variety Schwetzinger (R 1/48); both reached the top wire and remained healthy. It is not known yet whether this German variety is a "carrier."

Table III.

Scions of the variety Fuggles grafted on "carrier" stocks.

Stock		Scion (Fuggles)	
Name of variety	Ref. no.	Ref. no.	Total height reached (cm.)
Golden Cluster	R 1/57	R 1/62 *a*	Top*
,, ,,	R 1/57	R 1/62 *a*	375
,, ,,	R 1/58	R 1/62 *a*	300
Humphrey	R 1/66	R 1/63 *a*	Top
,,	R 1/66	R 1/63 *a*	380
,,	R 1/67	R 1/64 *a*	165
,,	R 1/67	R 1/64 *a*	260
Red Vine Canada	R 1/70	R 1/64 *a*	Top
L 21	OZ 59	R 1/61 *a*	Top
,,	OZ 59	R 1/61 *a*	Top
OP 21	S 55	R 1/63 *a*	Top
,,	S 55	R 1/63 *a*	340
,,	S 60	R 1/63 *a*	Top
,,	S 60	R 1/63 *a*	Top
L 40	CC 10	R 1/61 *a*	310
,,	CC 10	R 1/61 *a*	Top
458	458	R 1/62 *a*	Top
,,	458	R 1/62 *a*	Top
,,	457	R 1/62 *a*	Top
P 13	D 13	R 1/61 *a*	Top
,,	D 13	R 1/61 *a*	Top
OH 8	A 14	R 1/62 *a*	Top
,,	A 14	R 1/62 *a*	Top
,,	OW 20	R 1/62 *a*	145
Y 86	GG 13	R 1/61 *a*	Top
,,	GG 13	R 1/61 *a*	Top

* The top of the wirework varies between 12 and 14 feet, *i.e.* between 3·7 and 4·3 metres, from the ground.

It has already been shown (11) that scions of the variety Fuggles when grafted on a hop plant severely affected by mosaic disease can grow vigorously, remain healthy, and produce hops which ripen their seeds normally. It has now been shown that similarly the Fuggles variety is not infected when grafted on "carriers."

Summary.

1. When scions of varieties susceptible to mosaic disease were grafted on German, American, Danish, English and Seedling varieties, they developed typical mosaic disease. It appears, therefore, that "carrier" varieties are common amongst commercial varieties of hops. Certain male hops also proved to be "carriers."

2. In a few cases susceptible scions grafted on "carrier" stocks remained healthy. These anomalous cases require further investigation.

3. As a rule, the "carrier" varieties show no trace whatever of mosaic disease; the leaves are of normal colour and the plant shows normal growth with high fertility.

4. A case is recorded where one "carrier" variety (Red Vine Canada) exhibited in one season nascent symptoms of disease. The term "masked mosaic" is suggested for the appearance shown.

5. Scions of the commercial variety Fuggles, when grafted on "carrier" plants, invariably remained healthy.

We take this opportunity of thanking Mr Alfred Amos, Spring Grove Farm, Wye, for his kindness in giving us every facility for obtaining scions from his hop garden.

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STUDIES ON POTATO VIRUS DISEASES

VI. FURTHER EXPERIMENTS WITH THE VIRUS OF A POTATO MOSAIC UPON THE TOBACCO PLANT

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(With Plates XIV–XVII and 1 Text-figure.)

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1. Introduction.

In a previous communication in this series[1], a description was given of some preliminary experiments with the virus of potato mosaic upon the tobacco and of the resulting ringspot disease in that plant. Experi-

[1] Smith, Kenneth M. (1929). Studies on Potato Virus Diseases. IV. *Ann. App. Biol.* xvi, No. 1.

mentation has been continued, and in the present paper some further facts concerning the behaviour of tobacco ringspot under varying conditions are presented. In the very large numbers of different inoculations described in this paper, where inoculation by both needle and aphis has become a routine, the writer has not considered it necessary to give details of numbers of plants used, temperatures, incubation period of the virus, etc.

Acknowledgment is due to Mr F. T. Brooks and Dr D. Keilin with whom the writer has discussed some of this work.

2. PRODUCTION OF AN INCREASE IN VIRULENCE OF THE VIRUS BY PROGRESSIVE NEEDLE INOCULATION OF TOBACCO RINGSPOT.

It has been shown already[1] that progressive needle inoculation of one strain of tobacco ringspot through successive tobacco plants resulted in a greatly increased virulence. Experiments have been conducted with four separate strains of ringspot, primarily induced as before by needle inoculation of tobacco with mosaic potato, var. Arran Victory, two in Virginia and two in White Burley. These viruses were passed through a series of tobacco plants by means of needle inoculation, and in every case a marked increase in virulence resulted. This virulence was such as to cause the death of very young tobacco plants in every case, and to produce a very severe necrosis in older seedlings. The behaviour of older seedlings so inoculated was fairly constant, the necrotic disease killed all the plant except the young central shoot. After a period of two to three weeks, the plant commenced to grow again (Plate XIV, fig. 1) and new leaves were formed, upon which a severe and sometimes brilliant mosaic-like mottling developed. Increase in virulence becomes apparent at the 4th or 5th successive inoculation. In the case of Virginia, at the 3rd or 4th series large numbers of double, more rarely treble, concentric rings appear in clusters around and along the needle scratches (Plate XIV, fig. 2). As the inoculations progress, the rings lose their clear-cut concentric form and merge through necrotic spots into large lesions (Plate XIV, fig. 3), until a point is reached where the degree of virulence is such that, especially in White Burley, the leaves die so rapidly after the appearance of the first symptoms, that neither rings nor lesions have time to develop before the whole leaf collapses and dies. The first symptom in the development of the most virulent type of disease, that is at the stage when actual rings are no longer formed, is fairly constant. It takes

[1] Smith, Kenneth M. *Loc. cit.*

the form of a very marked "clearing" of the veins, in which the veins of the younger leaves become bright yellow and necrotic and stand out in a strongly defined network. Following upon this the veins of the older leaves except the inoculated ones also stand out. Clear window-like patches appear in the younger leaves which rapidly grow limp, shrivel and disappear. Within a few days of the appearance of first symptoms, all the outer leaves are dead and the central shoot alone is left alive, and in some cases this also is killed. As a rule however in older plants the central shoot persists, and later commences to grow again. Apart from the formation of actual rings "clearing" of the veins is a most constant and characteristic first symptom, and it is an invariable first symptom of the aphis-produced disease when so transmitted from ringspot tobacco to healthy tobacco. It is often the case with young plants that the *inoculated* leaves remain green and apparently living until the very end, long after the younger leaves have disappeared. The only symptom which appears to develop upon the inoculated leaves is the double concentric ring, and that usually when the inoculations have reached the 3rd or 4th plant. Plate XVII, fig. 1 shows the type of lesion which develops in White Burley tobacco. During these progressive needle inoculations therefore, the virus exhibits a fairly constant sequence of symptoms. Starting with the double rings produced by direct inoculation from mosaic potato, as inoculation progresses these rings become more pronounced and necrotic, gradually losing their sharp outline and joining together into irregular groups. Later comes the production of the intensified clearing of the veins, followed by clear window-like lesions, the whole leaf hanging limply without turgidity. It is at this stage of the progressive inoculations that the plant dies down and remains stationary for some weeks. Growth then recommences and what is virtually a new plant is formed. On the new leaves thus formed, the symptoms enter upon another phase; both rings and necrotic lesions have usually disappeared and their place is taken by mottling of a most pronounced type, which is occasionally sufficiently brilliant to simulate ordinary tobacco mosaic (Plate XVII, fig. 2). There may develop also white, yellow, or dark green patches, while occasionally very heavy lines of dark green may appear along the veins. What may be the significance of this sequence and variety of symptoms is not at present known. It might be suggested that the virus itself is undergoing some transformation, and these successive symptoms represent transitional stages in such a transformation, or they may represent merely a process of adjustment between the plant host and the accentuated virus,

whereby the plant is finally enabled to continue its growth, although the virus is still present in the sap.

3. COMPARISON OF NEEDLE AND APHIS (*M. PERSICAE*) INOCULATIONS OF HEALTHY POTATO FROM A TOBACCO PLANT AFFECTED WITH THE VIRULENT TYPE OF RINGSPOT.

Needle inoculation of healthy potato plants with the ordinary form of tobacco ringspot produces a mosaic in which symptoms are intensified and infective power greatly increased. Similar inoculation into potato with the virulent form of tobacco ringspot, that is, ringspot passed through a number of succeeding tobacco plants, produces an additional factor. On the young leaves of a potato plant so inoculated appear first the symptoms of the intensified mosaic, characteristic of ordinary ringspot inoculation, later however the lower leaves of the potato plant commence to die and death proceeds rapidly up the plant, the leaves first turning yellow and then shrivelling completely. The whole plant may finally die (Plate XV, figs. 1, 2). Death of the whole plant however does not always take place, often the lower leaves only die; just as in the case of tobacco affected with the virulent virus death may be confined to the older leaves of the plant. A series of needle inoculations was made from a potato plant, which was dying from the effects of the virulent ringspot virus, back into healthy White Burley tobacco seedlings. These developed the typical virulent form of the disease and rapidly died, a parallel series of inoculations from another potato similarly inoculated in another experiment produced the same effect on the Connecticut variety of tobacco. This seems to show that passage through healthy potato does not involve any marked change in the virulent virus. Aphides have been induced to carry infection with regularity to healthy potato from tobacco affected with the virulent ringspot. The disease resulting in the potato was symptomatically the same as that produced by aphis infection from ordinary ringspot tobacco, *i.e.* an intensified form of mosaic. When this disease was returned to healthy tobacco seedlings by needle, the dark green lines typical of aphis infection were produced. Comparison of the respective inoculations of healthy potato by aphis and needle from tobacco affected with the virulent virus shows that the lethal character of the disease is not transmitted by the aphis.

4. Comparison of needle and aphis (*M. persicae*) inoculations of healthy tobacco from tobacco plants affected with ringspot in both its ring-like and its virulent form.

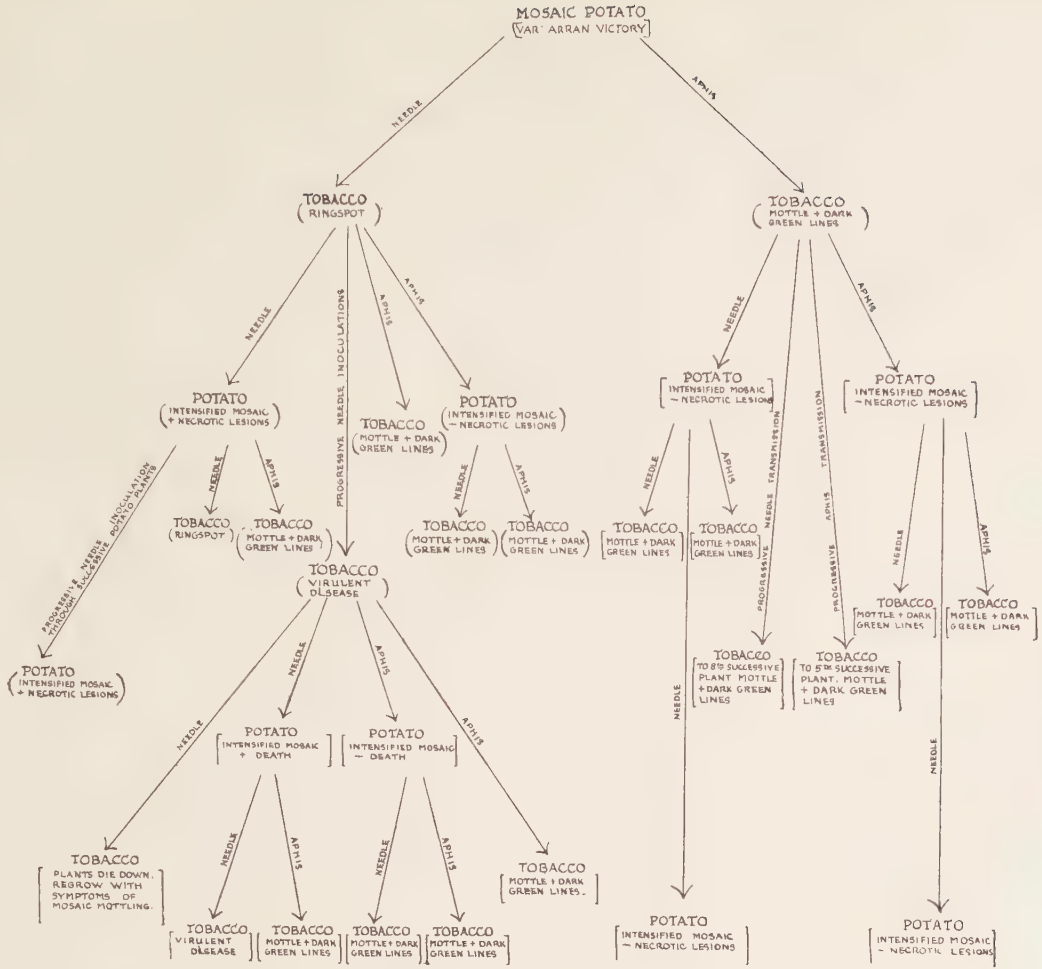
It has been shown[1] that needle and aphis (*M. persicae*) inoculations respectively from a mosaic potato into tobacco or from ringspot tobacco into healthy tobacco produce two diseases symptomatically different. Further experiments on this subject have been performed using as sources of infection ringspot tobacco plants exhibiting the disease in both ring-like and virulent forms. As regards aphis transmission of ordinary ringspot, a very large series of experiments has been performed. Aphis transmission has been carried out on six varieties of tobacco, *i.e.* Virginia, White Burley, Connecticut, Havana, Maryland and Kentucky. These six varieties were used in the experiments both as healthy plants to be infected and as sources of infection. All six varieties developed the characteristic clearing of the veins as a first symptom on the young leaves, followed by the appearance of the typical dark green lines or spots, mostly along the veins, which is the invariable appearance of the aphis-induced disease from a ringspot source (Plate XV, fig. 3). It was also found possible to pass the aphis-induced disease through potato and back to tobacco by the needle where it still retained its original character (Plate XV, fig. 4). This seems fairly conclusive evidence that the aphis-induced disease is not merely a varietal reaction on the part of one particular kind of tobacco. The variety Virginia is very resistant to aphis infection but can be inoculated by means of the needle with the aphis-induced disease from another variety. Further experiments were performed using a tobacco plant affected with the virulent virus as the source of infection. Needle inoculation from this plant produced a similar though slightly more severe disease in the inoculated plants, while aphis inoculation from the same source produced the characteristic green lines. This experiment is illustrated in Plate XVI, figs. 1, 2, 3. That this aphis-induced disease is a definite virus of an infectious nature and constant character has been proved experimentally. Once induced in tobacco by the aphis (*M. persicae*) it is possible to transmit it to other tobacco plants by means of both needle and aphis, and the symptoms remain constant in the inoculated plants. This disease has been needle-inoculated from a tobacco plant in which it was originally induced by the aphis, through eight succeeding tobacco plants (Plate XV, fig. 3), the symptoms remaining constant throughout the series, *i.e.* clearing of the veins followed by the development of the typical mottling and lines of

[1] Smith, Kenneth M. *Loc. cit.*

darker green. Little, if any, increase in the intensity of the symptoms was apparent. A parallel series of progressive inoculations was carried out using the aphis instead of the needle, and was carried to the 5th series of plants; the same result was apparent here except that the disease showed a tendency to attenuation in the last series. There are one or two possible suggestions for this production of two apparently distinct virus diseases from a single source of infection. It cannot be that the aphis picks up one constituent part only of a possible virus complex, because it has been shown already that *M. persicae* can transmit potato mosaic to healthy potatoes unchanged. It may be that the difference in symptoms is due to the different mode of inoculation. If this were the case, however, it should hardly be possible to pass on the aphis-induced disease unchanged by means of the needle. In this connection an attempt was made to simulate aphis inoculation of tobacco by pricking the leaves with a fine needle, lightly dipped in the virus, about a dozen fine punctures being made in the leaves of each plant. A series of White Burley plants was inoculated in this manner, using a strain of virulent virus. The disease produced, however, was of the ordinary severe type and not that characteristic of aphis infection. Another explanation may be that some substance in the sap of the tobacco plant reacts upon the saliva of the aphis and consequently upon the virus presumably contained therein. It is quite possible that in reality the actual difference between the aphis-induced disease and the various forms of tobacco ringspot is not so great as would appear from their symptoms, and further experiment may indicate the liaison existing between them. On one or two occasions a mottling disease very similar in appearance to the aphis-transmitted disease has resulted from needle inoculation from mosaic potato, notably some examples of mosaic in the variety President. This however is an exceptional occurrence. The most common result of needle inoculation of tobacco from mosaic potatoes of one or more varieties is the formation of the typical double rings or necrotic spots. Using the same tobacco plant affected with the virulent form of ringspot as source of infection it has been found possible to infect healthy tobacco seedlings with two separate diseases. Firstly, by inoculating the seedlings with the aphis the disease showing the characteristic dark green lines was produced, and then needle inoculation with the juice from the same source produced the virulent type of disease. The presence of the first or aphis-induced disease does not prevent the rapid development of the second or needle-induced disease.

5. Analysis of a comparative series of aphis (*M. persicae*) and needle inoculations with potato mosaic and tobacco ringspot.

In a previous communication a description was given of respective aphis and needle inoculations from mosaic potato to tobacco plants. It has been shown that such needle inoculations produced ringspot while inoculations with the aphis from the same source produced a mottling accompanied by lines and spots of darker green. In Text-fig. 1 an attempt has been made to portray diagrammatically the large numbers of needle and aphis inoculations which have been carried out with potato mosaic and tobacco ringspot and to show the resulting symptoms in each case. Considering the left half of the diagram first, it will be seen that beginning with mosaic potato, var. Arran Victory, needle inoculation from that source produces ringspot in tobacco. If that is passed by needle into potato an intensified form of the original mosaic is produced together with numbers of small necrotic lesions. Aphis transmission from the same ringspot to healthy potato produces an intensified form of mosaic very similar in appearance to that produced by the needle but lacking the necrotic lesions. Taking the needle-produced intensified mosaic in potato as the source of infection, needle inoculation of that into tobacco reproduces tobacco ringspot while aphis inoculation from the same source into tobacco produces the typical mottle accompanied by lines of darker green. Considering now the aphis-induced intensified mosaic in potato as the source of infection, needle and aphis inoculations from that into healthy tobacco both produce the same disease, *i.e.* the mottle accompanied by dark green lines. Next, reverting to the original ringspot tobacco, progressive needle inoculation of this virus through successive tobacco plants produces the virulent type of disease; incidentally it may here be pointed out that aphis inoculation from ordinary ringspot tobacco produces the typical mottle and dark green lines. Taking now as source of infection a tobacco plant affected with the virulent form of ringspot, further needle inoculation of this into tobacco kills all the outer leaves of the plant leaving the central shoot only alive, which after a week or so starts into new growth producing leaves upon which different symptoms of a mosaic type develop. Needle inoculation into healthy potato with the virulent form of ringspot produces first of all the intensified mosaic followed usually by rapid death of the plant. Needle inoculation from this plant back into tobacco reproduces the virulent ringspot; aphis inoculation from the same source produces the typical dark green lines disease. Considering now aphis transmission


MOSAIC POTATO
(VAR. ARRAN VICTORY)
NEEDLE
APHIS
TOBACCO
(RINGSPOT)
TOBACCO
(MOTTLE + DARK GREEN LINES)
PROGRESSIVE NEEDLE INOCULATIONS
POTATO
(INTENSIFIED MOSAIC + NECROTIC LESIONS)
TOBACCO
(MOTTLE + DARK GREEN LINES)
POTATO
(INTENSIFIED MOSAIC - NECROTIC LESIONS)
TOBACCO
(RINGSPOT)
TOBACCO
(MOTTLE + DARK GREEN LINES)
TOBACCO
(MOTTLE + DARK GREEN LINES)
TOBACCO
(MOTTLE + DARK GREEN LINES)
TOBACCO
(VIRULENT DISEASE)
PROGRESSIVE NEEDLE INOCULATIONS THROUGH SUCCESSIVE POTATO PLANTS
POTATO
(INTENSIFIED MOSAIC + NECROTIC LESIONS)
POTATO
(INTENSIFIED MOSAIC + DEATH)
POTATO
(INTENSIFIED MOSAIC - DEATH)
TOBACCO
PLANTS DIE DOWN. REGROW WITH SYMPTOMS OF MOSAIC MOTTLING.
TOBACCO
(VIRULENT DISEASE)
TOBACCO
(MOTTLE + DARK GREEN LINES)
TOBACCO
(MOTTLE + DARK GREEN LINES)
TOBACCO
(MOTTLE + DARK GREEN LINES.)
TOBACCO
(MOTTLE + DARK GREEN LINES)
POTATO
(INTENSIFIED MOSAIC - NECROTIC LESIONS)
TOBACCO
(MOTTLE + DARK GREEN LINES)
TOBACCO
(MOTTLE + DARK GREEN LINES)
NEEDLE TRANSMISSION
PROGRESSIVE APHIS TRANSMISSION
TOBACCO
TO 8TH SUCCESSIVE PLANT MOTTLE + DARK GREEN LINES
TOBACCO
TO 5TH SUCCESSIVE PLANT. MOTTLE + DARK GREEN LINES
POTATO
(INTENSIFIED MOSAIC - NECROTIC LESIONS)
TOBACCO
(MOTTLE + DARK GREEN LINES)
TOBACCO
(MOTTLE + DARK GREEN LINES)
POTATO
(INTENSIFIED MOSAIC - NECROTIC LESIONS)

Text-fig. 1.

from the tobacco affected with the virulent form of ringspot; first into potato, a similar intensified mosaic is produced as by needle but lacking its lethal character. Aphis and needle transmission from this last source into tobacco produces in each case the typical dark green lines in the inoculated plant. Aphis inoculation of the virulent form of ringspot into healthy tobacco produces the same dark green lines as are produced by aphis inoculation from the ordinary form of ringspot into healthy tobacco. That concludes the left-hand half of the diagram where the inoculations had a common source of infection in needle-induced ringspot of tobacco. In the right-hand half of the diagram the common source of infection is a tobacco plant with the mottle and dark green lines disease induced by aphis transmission from mosaic potato. Needle and aphis inoculations respectively from this tobacco plant into healthy potato produced the same intensified mosaic in each case. This disease was symptomatically identical with the disease produced by aphis inoculation of potato from ordinary ringspot tobacco, and differed apparently from that produced by needle inoculation from ringspot tobacco in that the small necrotic lesions were absent. Reverting to the intensified mosaic produced in potato by both needle and aphis from the aphis-infected tobacco, needle and aphis inoculations of both these diseases back into tobacco produced in all four series the same mottle and dark green lines as were exhibited by the tobacco which was the original source of infection. In the centre of the right-hand half of the diagram are represented progressive needle and aphis inoculations respectively from the same source of infection through succeeding tobacco plants.

The progressive needle inoculations were carried up to the 8th plant (Plate XV, fig. 3) and the progressive aphis inoculations up to the 5th plant. Little, if any, increase in virulence was noted in either case, possibly the symptoms were somewhat brighter towards the end of the needle sequence, while a tendency to attenuation was apparent in the case of the aphis. In reviewing the inoculations set forth in Text-fig. 1, it will be seen that most of the possible cross inoculations have been carried out. Perhaps the outstanding fact arising from these comparative inoculations is the constant character of the aphis-induced disease; wherever an aphis transmission enters into a series, inoculation into tobacco subsequent to that, either by aphis or needle, produces the mottle and dark green lines disease. The respective symptoms arising from the two methods of transmission most nearly approach a common point of similarity in the potato plant. The text-figure shows that transmission to potato from tobacco either by needle or aphis produces the intensified

form of mosaic whether the source of infection in the tobacco be induced by needle or aphis. The apparent difference between the needle- and aphis-induced mosaics in potato lies in the production of small necrotic lesions when the disease is needle-transmitted from ringspot tobacco, and also in the subsequent death of the potato plant (Plate XV, figs. 1, 2) when the needle transmission is effected from a tobacco with the virulent disease. It should be noted however that in the case of needle and aphis infections of healthy potato from tobacco affected either with ringspot or the "aphis disease," the intense mosaics produced are really very similar, and if, as sometimes happens, the small necrotic lesions are absent, then symptomatically the diseases are identical. Yet in spite of this close resemblance, the text-figure shows that they do not behave similarly when returned to tobacco. In all the inoculations so far carried out, a *needle*-produced mosaic in potato from ringspot tobacco when returned to tobacco by needle reproduced ringspot or spot-necrosis, while an *aphis*-produced mosaic in potato either from a ringspot tobacco or an aphis-infected tobacco when returned to tobacco by aphis or needle produced the typical dark green lines.

It would appear therefore that the aphis does not transmit some element of the virus which is concerned with the production of the more necrotic and lethal symptoms.

6. Response of different varieties of tobacco to needle inoculation with the virulent virus.

Further experiments have emphasised the greater resistance of Virginia tobacco over White Burley. In a series of needle inoculations of the virulent virus made into twelve White Burley and twelve Virginia plants, all small seedlings of the same age and size, the White Burley plants all died, but half the Virginia plants partially recovered and survived for some months albeit severely infected. Again, it has been found many times that a strain of virus, which on Virginia still produces double rings, produces severe necrotic lesions when inoculated into White Burley. The age of the plant at the time of inoculation also governs to some extent the degree of severity of the resulting disease. Very small seedlings of White Burley when inoculated with the virulent form of the virus invariably die, but half-grown plants, on the other hand, often survive and may exhibit a less severe form of the disease. Four other varieties of tobacco were tested, Kentucky, Maryland, Havana and Connecticut, and showed a susceptibility more comparable to that of White Burley. They were all less resistant than Virginia.

In the case of needle inoculation into Virginia with a strain of virulent virus, it often happens that the plant manages to grow away from the disease to a certain extent. Such a plant may live for months and even flower and set seed. It exhibits the typical severe lesions on the lower leaves, but the upper part of the plant appears normal, the young leaves being green, symptomless and apparently healthy. A series of parallel inoculations was made into healthy tobacco seedlings from these green leaves, and into other seedlings from the older leaves which showed lesions. All the inoculated plants developed the disease to the same degree of virulence, thereby showing that the virus was still active in the green apparently normal leaves. Although the degree of virulence of the virus was equal in both green and diseased leaves, it does not always follow that this virulence is necessarily as great as at the time of original inoculation into the plant.

7. Non-transmission of ringspot through the seed.

Seed was saved from twelve tobacco plants of the two varieties, Virginia and White Burley, affected with ringspot, including the virulent form and the aphis-induced disease. In all about 500 seedlings were grown; all proved healthy. This experiment seems to show that ringspot is not commonly, if at all, transmitted by the seed, and thereby it falls into line with other virus diseases of plants. Subsequent inoculation of these plants with ringspot produced the disease, there being no difference in their reaction from that exhibited by seedlings from healthy plants.

8. Distribution of the virulent virus in an affected tobacco plant.

The leaves of six White Burley seedlings were needle-inoculated with an inoculum made from the roots of a tobacco plant affected with the virulent virus. In ten days all six plants developed the normal virulent type of disease, thus showing that the virus is present in the roots of affected plants. The converse of this experiment was also performed, the roots of six healthy White Burley plants were inoculated with an inoculum made from a leaf of a tobacco plant affected with the virulent virus. In 14 days these six plants developed the virulent type of disease.

9. ATTENUATION OF THE VIRULENT VIRUS BY AN APPARENT INHERENT RESISTANCE IN CERTAIN INDIVIDUAL PLANTS.

Large numbers of needle inoculations into tobacco plants have shown that an average of one plant in six, even a smaller average of the susceptible variety White Burley, possesses what is presumably an inherent resistance to the virulent ringspot virus. Such plants, instead of developing the disease in its severe form, show weaker symptoms which often exhibit a tendency to return to ring formation. Experiments were made in order to determine whether this phenomenon was due to a definite attenuation of the virus by some inherent character in such individual plants, or whether the plant was in some way more tolerant of the virus and so was acting as a partial "carrier." A series of needle inoculations was made from such a plant (Virginia) into healthy Virginia and White Burley seedlings. The Virginia plants developed a mild type of disease very similar to that exhibited by the source of infection, the White Burleys developed a disease more severe than in the Virginias, but much less severe than was produced in similar White Burley plants by the same strain of virus which had not been passed through the Virginia. That the White Burleys would be more seriously affected than the Virginias was to be expected in the light of the greater susceptibility of the former. This experiment seems to suggest that the virus is definitely attenuated by its passage through plants possessing this apparent inherent resistance. That this inherent resistance is easily broken down is shown in the succeeding section. This phenomenon should be distinguished from that described in section 6 where a plant has developed the virulent form of the disease and then has partly recovered. In such a plant, although the young leaves show no symptoms, the virus seems to retain much of its original intensity. In reviewing the above experiments the possibility must be considered that this attenuation of the virus may be due, not to an inherent resistance in individual plants, but to the reception by such plants of minute quantities only of the virus, by accident of inoculation, and that such minute quantities of the virus are capable of causing an attenuated, though constant, form of the disease.

10. Some preliminary attempts to develop in tobacco plants an immunity to the virulent virus.

For this work the susceptible variety White Burley was used, and the first experiment was as follows: six White Burley plants were needle-inoculated with a strain of the virulent virus which had been killed by heating over a spirit lamp. After 21 days, no symptoms having developed, the plants, together with three other White Burleys to serve as controls, were inoculated with the same strain of virus unheated. In 11 days all the plants developed the ordinary virulent form of the disease, there being no apparent difference between the plants treated with the "dead" virus and the control plants. In the next experiment a number of tobacco plants were selected which had already been inoculated with the most virulent form of the virus but owing to some inherent resistance, the symptoms developed had not been of the severe necrotic character usually exhibited by this virus but had appeared in a modified form of ring-like tendency, severe enough in some cases but not sufficiently so to interfere with the growth of the plants. Twelve plants in all were selected for this experiment, and they were needle-inoculated a second time with the same strain of virus. Half the plants were given the virus from the same sources from which they had already received it, and half from affected plants later in the sequence of successive inoculations; these latter would therefore receive a virus possibly slightly more virulent in character. In ten days all twelve plants developed the virulent form of disease, the typical yellowing of the veins appearing on the youngest leaves and later on the older ones. Clear patches then developed on the leaves which lost all turgidity and hung limply like thin cloth. Of these plants only the central shoot was left alive; later this commenced new growth, the new leaves exhibiting a marked mosaic-like, though still necrotic, type of mottling. It is thus apparent that, under certain conditions, it is possible to inoculate a tobacco plant twice with the same virus, if there exists some factor of resistance to the first inoculation. In addition the experiment shows that the inherent resistance is easily overcome, and also that the presence of the virus in an attenuated form in the plant is no bar to the rapid development of the virulent form of the virus and confers no immunity to later infection. In a further experiment an attempt was made to ascertain the result of double inoculation of tobacco plants, where the first inoculation consisted of the virulent virus artificially attenuated by alcohol. The first series consisted of six

White Burley seedlings inoculated with a suspension of the virus in 75 per cent. alcohol, the leaf from the source of infection being ground up with the alcohol and inoculated immediately into the plants. The typical virulent disease developed in 14 days and killed the seedlings. The experiment was then repeated keeping the virus in 75 per cent. alcohol for varying periods until the symptoms produced in the inoculated plants were very faint, showing that the virus had been attenuated by the alcohol. Subsequent inoculation of these plants with virus free from alcohol produced the typical virulent disease, thus showing that no immunity had been conferred by the attenuated virus. The last experiment in this series deals with the double inoculation of a tobacco plant from the same source of virulent ringspot but using the aphis as the first method of inoculation. Six plants of White Burley, affected with the typical dark green lines disease induced by means of aphis from a plant with virulent ringspot, were needle-inoculated from the same source. The disease developed normally, the symptoms of both diseases being present together in the same plant. These attempts to produce an immunity in tobacco are only of a preliminary nature, but so far as they go they offer little hope that such an immunity can be experimentally conferred.

11. Behaviour of the progeny of potato plants inoculated with tobacco ringspot the preceding year.

Numbers of tubers from potato plants (vars. Arran Victory and President) needle-inoculated in 1928 with tobacco ringspot were planted in 1929. The resulting plants showed the same intense mosaic, accompanied by small necrotic spots, as was exhibited by the parent plants the preceding year. Needle inoculation from these plants into Virginia tobacco produced typical ringspot. Into White Burley spot necrosis was produced in some cases and, in others, double rings along the inoculation scratches. In this last respect the symptoms differed from those produced by direct inoculation into White Burley with ordinary potato mosaic, as double rings do not usually form along the scratches on a first inoculation from ordinary potato mosaic to tobacco. Occasionally, also, juice from potato plants arising from parents affected with the intensified form of mosaic produced severe necrosis in White Burley tobacco plants. Juice from these potato plants was also inoculated into healthy Arran Victory, symptoms of the intensified mosaic developing in nine days, thereby showing that the increased infective power was still retained.

These facts seem to indicate that the change in the virus of potato mosaic induced by its passsage through tobacco may be of a permanent nature.

12. Discussion.

By improvements in methods of technique the writer has induced the aphis, *M. persicae*, to carry infection with fair regularity from infected tobacco to healthy potato. As is shown by Text-fig. 1, it has been found possible to infect potato (vars. Arran Victory and President) with the intensified mosaic by *M. persicae* from tobacco affected with ringspot, the spot necrosis form, the aphis-induced disease, and the virulent form induced by progressive inoculation. All these diseases thus aphis-transmitted to potato have produced identical symptoms, and the mosaics produced have also behaved similarly when returned to tobacco by needle or aphis. The chief points in the behaviour of tobacco ringspot when produced by needle inoculation from mosaic potato, and of the mottle and dark green lines disease produced by aphis inoculation also from mosaic potato, may be briefly compared. Firstly ringspot can be greatly increased in virulence and infective power by progressive needle inoculation through successive tobacco plants. When needle-inoculated into potato in its ringspot form, a mosaic is produced with enhanced symptoms and increased infective power; when inoculated into potato in its virulent form the same mottling is produced, but is usually followed by the death of the whole or part of the potato plant. When these forms are returned to tobacco by the needle, they produce substantially the same disease as was shown before passage through potato. Comparing, then, this behaviour with that of the disease produced by aphis from mosaic potato, progressive inoculation through succeeding tobacco plants either by aphis or needle does not produce increase in virulence, at least when taken as far as the 8th successive plant. Return of this disease to healthy potatoes, either by needle or aphis, produces apparently the same intensified mosaic as was produced by needle inoculation with the ringspot form but lacking the necrotic lesions usually present with the latter. When returned to tobacco by aphis or needle the same disease is produced as was present before passage through potato.

It will be seen then that both the needle- and aphis-produced diseases are fairly constant in their behaviour. The change of symptoms, whatever it may portend, which is produced by the aphis is apparently permanent, as shown by the behaviour of the disease when subsequently passed on

by the needle through either potato or tobacco. On the other hand, the close similarity between the symptoms produced in potato by the two diseases seems to indicate that the difference between them is in reality only slight. It is difficult at the present stage of the work to do more than state the facts as they are, trusting to later research to throw light on the true meaning of these apparent transformations of the virus.

13. SUMMARY.

Further experiments with the virus of a potato mosaic upon the tobacco plant are described.

(1) Marked increase in virulence has been shown to occur in ringspot by progressive needle inoculation through succeeding tobacco plants. Four strains of ringspot arising from four distinct mosaic potato plants (var. Arran Victory) have been so experimented with in White Burley and Virginia tobaccos.

(2) Comparison of needle and aphis (*M. persicae*) inoculations of healthy potato plants from a tobacco plant affected with the virulent ringspot shows that although both methods of inoculation produce an intensified mosaic in the potato plant, that produced by the needle is often fatal to the potato.

(3) Comparison of needle and aphis (*M. persicae*) inoculations of healthy tobacco plants from a tobacco plant affected with the virulent ringspot shows that while the needle reproduces the virulent disease, the aphis produces a mottle and dark green lines disease, apparently the same as that produced by aphis transmission from the ordinary type of ringspot.

(4) An analysis is given of a large comparative series of aphis and needle inoculations with potato mosaic and tobacco ringspot. This indicates that the aphis-induced disease is an infectious virus of constant character. The experiments also show that the aphis fails to transmit the more lethal characters of the disease.

(5) The response of different varieties of tobacco to the virulent virus was examined. Six varieties were used in the experiments: White Burley, Virginia, Kentucky, Maryland, Havana, Connecticut; Virginia was found to be the most resistant, and White Burley the most susceptible, while the other four varieties approximated to White Burley in susceptibility.

(6) Large numbers of seeds were sown which had been derived from tobacco plants affected with ringspot and its various modifications. In no case was the disease transmitted through the seed.

(7) Experiment showed that the virulent virus was present in the roots of an affected tobacco plant; and also that needle inoculation of the roots of healthy tobacco plants with the virulent virus produced the typical disease.

(8) It was found that certain individual tobacco plants, both Virginia and White Burley, seem to possess an inherent resistance to the virulent virus which attenuates it. Needle inoculation of such a plant with the virulent virus produces faint symptoms only. When juice from this plant is passed on to other tobacco plants the virus still shows weak symptoms, as compared with those produced by the same strain of virus before its passage through such a resistant plant. This seems to indicate that certain plant individuals have the power of attenuating the virus.

(9) A description is given of some preliminary attempts to produce experimentally in tobacco plants an immunity to the virulent virus. Plants were first inoculated with a suspension of "dead" virus, killed by heating, and three weeks later they were inoculated with the same strain of virus unheated. Experiments were also performed giving, first, inoculations with the virus artificially attenuated, and then inoculating with the virulent virus. Tobacco plants affected with the aphis-produced disease were also needle-inoculated with the virulent virus. A series of resistant individual plants which had developed only a mild disease after inoculation with the virulent virus, was inoculated a second time, with the same strain of virus in some cases and in others with a different strain. In all the above experiments the normal virulent disease developed, no immunity having been conferred.

(10) Tubers, from potato plants infected the previous year with tobacco ringspot, were grown in order to study the behaviour of the virus after a winter spent in the resting tuber. Plants were produced exhibiting all the symptoms of the intensified mosaic characteristic of infection with tobacco ringspot which were exhibited by the parent plants the year before. This seems to indicate that the change induced in the virus of a potato mosaic by passage through tobacco may be of a permanent nature.

Fig. 1.

Fig. 2.

Fig. 3.

SMITH.—Studies on Potato Virus Diseases (pp. 382–399).

Fig. 1.

Fig. 2.

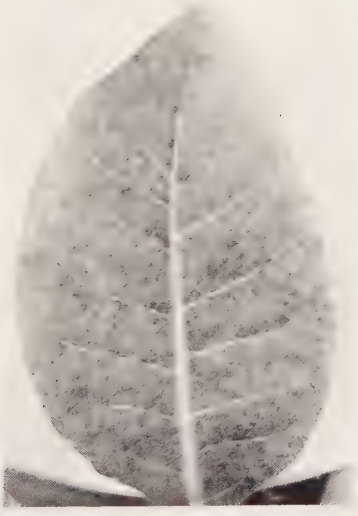


Fig. 3.

Fig. 4.

SMITH.—Studies on Potato Virus Diseases (pp. 382–399).

Fig. 1.

Fig. 2.

Fig. 3.

SMITH.—Studies on Potato Virus Diseases (pp. 382–399).

Fig. 2.

Fig. 1.

SMITH.—Studies on Potato Virus Diseases (pp. 382–399).

EXPLANATION OF PLATES XIV—XVII

PLATE XIV.

Fig. 1. White Burley tobacco plant starting into new growth, after inoculation with the virulent form of ringspot. Note the old leaves killed by the virus.

Fig. 2. Rings forming along the needle inoculation scratches on the leaf of tobacco, var. Connecticut, fourth successive inoculation.

Fig. 3. Leaf of Virginia tobacco plant, showing the severe lesions produced by the virulent virus. Length of leaf 12 cm.

PLATE XV.

Fig. 1. Potato plant, var. Arran Victory, needle-inoculated with the virulent virus from tobacco, showing death of the lower leaves.

Fig. 2. Potato plant, var. Arran Victory, killed by needle inoculation with the virulent virus from tobacco.

Fig. 3. Leaf of White Burley tobacco showing the typical appearance of the aphis-induced disease. This particular plant was the eighth in a series of successive needle inoculations of the disease, after it had once been produced in tobacco by the aphis. No appreciable increase in virulence was obtained.

Fig. 4. Leaf of White Burley tobacco affected with the aphis-induced disease. In this case the disease, after production in tobacco by the aphis, had been needle-inoculated through potato and back to tobacco. The symptoms remained unchanged.

PLATE XVI.

The three figures in this plate show comparative needle and aphis inoculations into tobacco from the same source of infection.

Fig. 1. The source of infection, a White Burley tobacco plant affected with the virulent virus.

Fig. 2. A White Burley tobacco plant which has been needle-inoculated from the plant shown in Fig. 1.

Fig. 3. Leaf of a White Burley seedling aphis-inoculated from the plant shown in Fig. 1. The development of the dark green lines is plainly shown.

PLATE XVII.

Fig. 1. Leaf of White Burley tobacco showing the large lesions produced by the virulent virus.

Fig. 2. Leaf of White Burley tobacco plant showing the mosaic-like mottling sometimes produced by the virulent virus, after the plant has partially recovered from the disease.

Photographs by C. W. Williamson.

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DALDINIA CONCENTRICA ATTACKING THE WOOD OF *FRAXINUS EXCELSIOR*

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(With 23 Text-figures.)

An English timber merchant, on cutting up a butt of an ash tree, found that the wood was beautifully marked with irregular, brown to black, bands, lines and patches, yet the wood was sufficiently firm to permit of its being made into ornamental boxes. Such wood is so far from being unfamiliar to timber merchants that, according to Mr J. Richardson, it has the name of "calico-wood."

External appearance of "calico-wood."

The wood was streaked with irregular, wavy, purplish-brown to black stripes, which were not confined to any one zone of the annual rings but

Fig. 1. "Calico-wood" cut longitudinally, showing the dark streaks and the blackened pore zone.

ran along and across them; these lines were usually 1 to 5 mm. in width, but in some longitudinal or transverse sections they appeared as circular patches or rings. The large spring vessels of every annual ring had black contents (Fig. 1).

ANATOMY OF NORMAL ASH WOOD.

Before describing the microscopical structure of this discoloured wood, a description of normal ash wood will be given.

A normal annual ring of *Fraxinus excelsior* is made up of: (1) on the inside a zone of spring wood, the pore zone, consisting of wide vessels surrounded by wood parenchyma, between which lie fibres with slightly thickened walls; (2) a wide middle zone with thicker-walled fibres, and scattered smaller vessels surrounded by wood parenchyma; (3) on the outside a limiting layer consisting of several rows of wood parenchyma with scanty, small, very thick-walled vessels. The spring vessels are very large compared with the size of the other elements; their walls bear numerous bordered pits, the canals of which are elongated owing to the wall-thickening, and widen suddenly into the cavity; the canals may be branched once or twice. The summer vessels are similar, becoming smaller and more thick-walled towards the outer part of the annual ring. The wood parenchyma is always associated with the vessels, surrounding the small ones on the inner and outer sides of the large ones. In the late summer wood, however, the parenchyma spreads away between the vessels in the form of an arc in transverse section. The wood fibres have simple pits and form the main groundwork of the wood; they are lined with a thin layer of protoplasm. There are also "intermediate fibres" which are transitional in structure between the wood parenchyma and the fibres; and their position is commonly near the true parenchyma. The medullary rays are numerous, 1 to 3 cells wide, 5 to 20 cells deep, the ray being lenticular in shape in tangential view; the inter-cellular spaces in the ray are well marked in tangential sections.

As regards the contents of the lumina, in my specimen all the cells of the wood parenchyma and of the medullary rays had colourless, yellow, or light brown, globular contents of an unknown composition, sometimes together with starch grains. No fats, tannins, or mucilages were found stored in the cells to any extent, but starch was contained in quantity and De Bary (1) states that in a stem of *Fraxinus* when 40 years old an abundance of starch was found by Gris in all the annual rings.

Anatomy of infected wood.

For microscopical examination the wood was cut into small blocks which were submerged in acetone and de-aerated, and then left in a solution of 12 gm. cellulose acetate in 100 c.c. acetone until soft enough (10–14 days) for cutting by microtome. The stains used on the sections were lactic blue, safranin and picro-aniline blue, and gentian violet.

In the wood, before artificially staining it, large patches of the section were brown, due to the change in colour of the wood walls and their contents. These patches had no defined limits, but included an indefinite number of cells, the colour being darkest in the centre of the patch and gradually fading out at the edges. In the walls, the pits contained a brown substance, and sometimes the middle lamella was the most deeply coloured, particularly near the pits and along the intercellular spaces, the rest of the wall taking on a lighter shade of brown. In transverse sections the brown colour extended farthest along the medullary rays, from a discoloured patch to the colourless wood. The cell-contents of the parenchyma were stained a deep brown (Figs. 2–5).

Fungal hyphae, present in large quantities, were of three kinds:

(1) Very fine, segmented, *colourless* hyphae with granular contents were seen when the sections were stained with lactic blue or picro-aniline blue. Branching was sparse and irregular, the hyphae as a rule were not matted together, but passed from cell to cell, through all the tissues, by means of the pits in the cell-walls. These hyphae were present in the brown and the colourless wood and occurred in the vessels, fibres, medullary rays and parenchyma cells.

(2) *Brown to black*, segmented, hyphae were visible in unstained sections. These were much wider than the colourless hyphae, and were of two kinds:

(*a*) In the large spring vessels these hyphae were *dichotomously branched*, short and brittle; the tips of the branches were pointed and at the junction of the branches the hypha was slightly swollen; there were no contents. This type of mycelium occurred in wood having brown or colourless walls, most abundantly in the spring vessels but also in the summer vessels, fibres and parenchyma cells. Discoloration of the ash wood was consequently due to the presence of this black mycelium as well as to the darkening of the walls.

(*b*) The other kind of brown hyphae were sparsely *branched in an irregular way*, wider than the dichotomous hyphae, and found almost exclusively in the medullary ray cells of the brown-coloured wood.

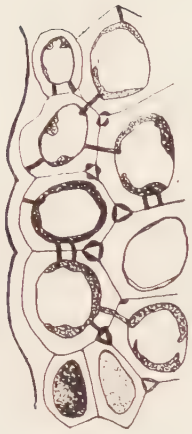


Fig. 2.

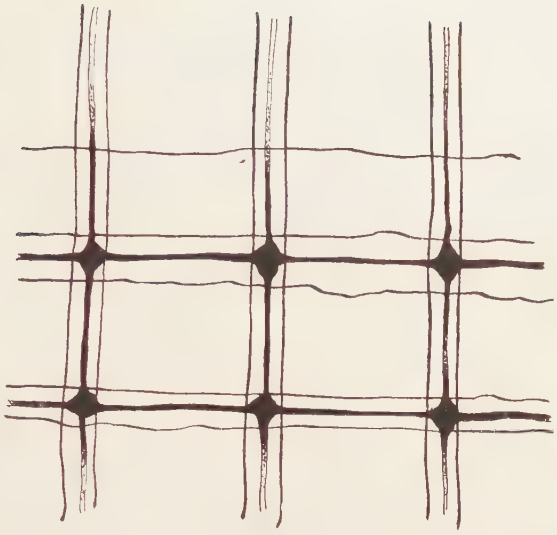
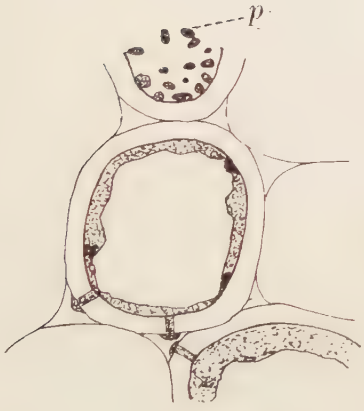


Fig. 4.



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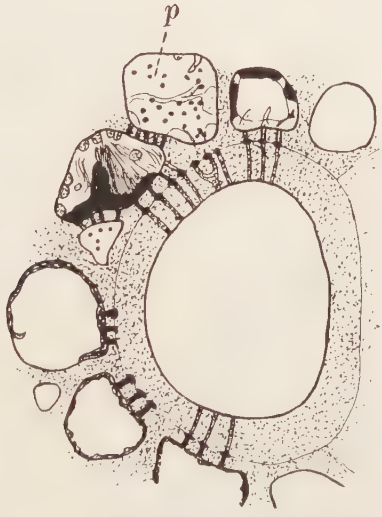


Fig. 5.

Fig. 2. Tangential longitudinal section of part of medullary ray, showing brown staining in pits.

Fig. 3. Part of Fig. 2 enlarged. p = pits in surface view.

Fig. 4. Radial longitudinal section, showing brown stain in middle lamellae of fibres and medullary ray cells.

Fig. 5. Transverse section of a small vessel and parenchyma, showing stain deepest in pits and paler in rest of wall. p = pits in surface view.

Fig. 6.

Fig. 7.

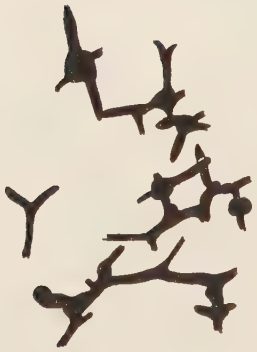


Fig. 8.

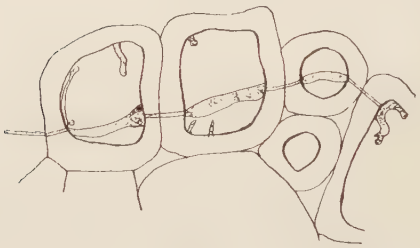


Fig. 9.

Fig. 6. Radial longitudinal section of medullary ray, showing straight brown hyphae in cells and passing through the pits.

Fig. 7. Transverse section of a vessel and parenchyma, showing black dichotomous hyphae (*d*) and colourless hyphae (*c*) in vessel.

Fig. 8. Black dichotomous hyphae from a vessel, enlarged.

Fig. 9. Transverse section, showing colourless hypha passing across the fibres *via* the pits.

Peg-like branches from these hyphae passed through the pits of the medullary ray cells, and the hyphae were empty (Figs. 6–9).

The general appearance of the contents of the parenchyma cells was not changed much by the presence of the fungus or of the brown colour, except that they were stained by the latter. The globular bodies described as occurring in the cells of normal ash were present in the brown and the colourless wood. Starch grains were scanty, but a few were seen in brown patches as well as in the colourless wood.

Lignin, pectin and cellulose occur in the walls of normal ash. The wood infected by this fungus remained firm, and the cell-walls did not show any change in thickness or structure except where the pits were perforated by the hyphae. Phloroglucin with hydrochloric acid was

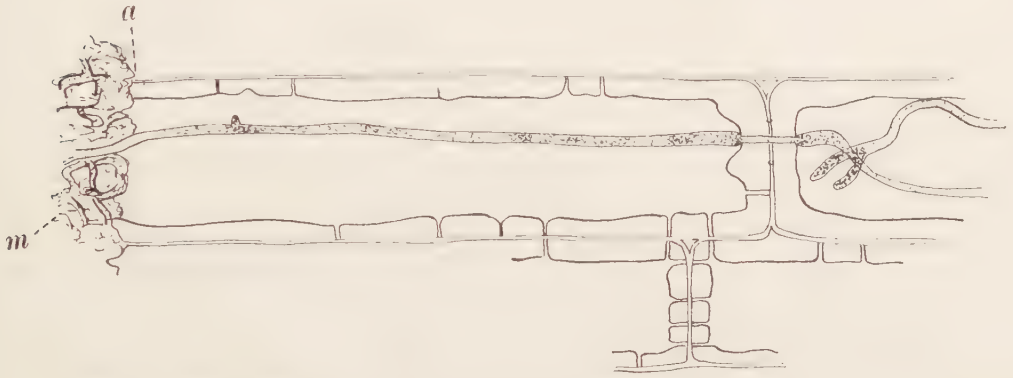


Fig. 10. Radial longitudinal section, showing a colourless hypha entering the cut end (*a*) of a medullary ray cell, after artificial infection of ash wood, and passing inwards into the next cell through a pit. *m* = mycelium outside the wood.

used to test the presence of lignin, ruthenium red and methylene blue the presence of pectin. These were found to occur in both colourless and brown woods, there being no complete disappearance of either substance in the infected wood.

From experiments, which will be described later, whereby ash wood was artificially infected with the spores of this fungus, the attack and development of the fungus in the wood could be understood. The fungus entered through the vessels, fibres or medullary rays at the cut end of a piece of wood. In the vessels, the hyphae on entering were very fine and colourless; they increased in number and became wider and tangled together; and they travelled down the vessel penetrating far into the wood. In the fibres, the hyphae passed straight down, rarely branching at first, but eventually branches were formed at right angles to the

original hypha, passing through the fibre pits into adjoining cells. In the medullary rays, the colourless hyphae travelled along the length of the ray, while some hyphae branched off and passed up and down the wood. These hyphae always travelled from one cell to another by means of the pits, thus permeating the whole tissue locally (Fig. 10).

With the ageing of the fungus the formation of black hyphae and the production of the brown colour ensue. In the artificial infections the black dichotomous hyphae were produced first near the cut end of the vessels, but eventually they occupied the whole length. They also were formed in the fibres and parenchyma cells, while the straight brown hyphae were produced in the medullary ray cells. Very soon after the hyphae had penetrated the wood, brown, amorphous, granular bodies were produced, most abundantly in the fibres and vessels, but also in the medullary ray cells and the wood parenchyma. It was concluded that the substance must be a product of decomposition of the nature of a "wound gum" as it was insoluble in acetone, alcohol, benzol, petrol ether, concentrated hydrochloric acid, nitric acid, sulphuric acid, potassium, ammonium and sodium hydroxide, but dissolved in a mixture of concentrated nitric and sulphuric acids. It did not stain with phloroglucin and hydrochloric acid, yet parts of it stained blue with lactic blue and picro-aniline blue (Fig. 11).

Fig. 11. Longitudinal section of fibres, showing granular bodies associated with artificial infection of ash wood.

Several problems present themselves as to the fungus responsible. First, the identity of the fungus or fungi present had to be determined; second, the conditions of activity of the fungus in wood, as regards water, oxygen and acidity, including the conditions of infection and germination, and the nature of the food used by the fungus; third, the origin and nature of the brown substance.

For the purposes of identification and of determination of the conditions of growth, and the effect of the fungal attack, a number of cultures of the fungus were made.

Cultures.

(1) In order to induce the fungus to grow out of the wood, a piece of the infected wood was soaked in running water for six hours or more, dipped in 0·1 per cent. mercuric chloride in alcohol, and then kept damp in a potato dish. In about a fortnight, conidial tufts emerged, forming small, irregularly branched, upright, pale pink hummocks, covered with spores. These tufts never grew out of the blackened spring vessels, but only from the colourless or brown summer wood.

The structure of these conidiophores and conidia agreed with that of *Daldinia concentrica*. Consequently, fruit-bodies of that fungus connected with the wood of living ash trees were obtained, so that the ascospores might be used for cultures.

(2) Small pieces of wood were sterilised by steam in large plugged test-tubes and a suspension of either the conidia or the ascospores in sterile, distilled water was added to the tubes. The woods used were: sapwood of sycamore, hornbeam, oak (conidia only); heartwood of mulberry, elm, walnut, pine (conidia only), larch and American birch (ascospores only); the ripewood of beech.

(3) The following synthetic media were infected with conidia and ascospores: (*a*) *solid media:* agar: plain, 2 per cent. peptone, 2–20 per cent. sucrose, 5–10 per cent. malt, 2 per cent. turnip, 2 per cent. potato, 2 per cent. prune, and 1 per cent. starch; gelatine: 2 per cent. bouillon, and 2 per cent. peptone; cotton wool + a solution of 1 per cent. KNO_3, 0·5 per cent. KH_2PO_4, 0·2 per cent. $MgSO_4$; (*b*) *liquid media:* tap water, distilled water, and solutions of different concentrations of malt, and sucrose; 2 per cent. galactose, 5 per cent. glucose, gum arabic, 1–5 per cent. starch, 0·5 per cent. onion pectin, 0·5 per cent. Citrus pectin, 0·5 per cent. beech hemicellulose B, 3 per cent. dextrose, 3 per cent. inulin, 2 per cent. xylose, 2 per cent. arabinose, 2 per cent. levulose, 2 per cent. maltose, 2 per cent. mannite and 0·5 per cent. lichenin.

(4) In order to show whether the spores needed an alkaline, acid or neutral medium in which to germinate, conidia and ascospores were placed in sucrose solutions containing different percentages of acid and alkali. The acids used were citric and acetic, and the alkali was caustic potash.

Identity.

(1) The conidiophores constituting the hummocks emerging from the moistened wood were hyaline, irregularly branched, septate; the branches bore verticillate branches, at the tips of which were produced clusters

of stalked conidia; the conidia were colourless, ovoid, with the attachment end more pointed, 6·5–8 × 5–6 μ in dimension. The type, as Bayliss Elliott (2) suggested, is evidently that of a *Botrytis* (Figs. 12, 13, 14, 15).

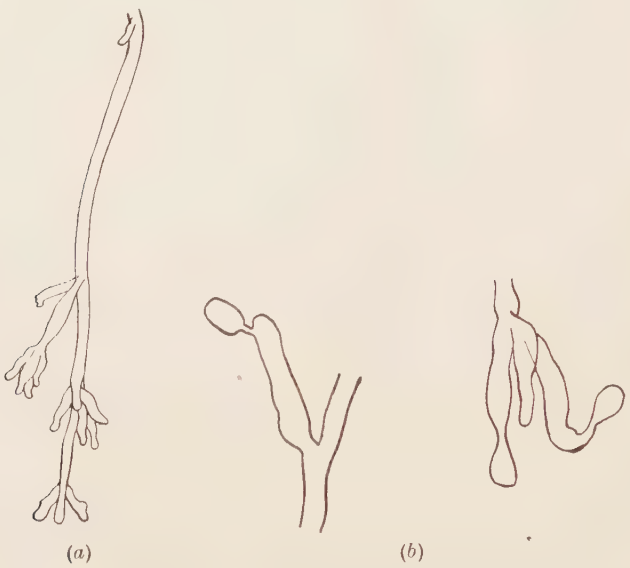


Fig. 12. (*a*) Conidiophore, and (*b*) verticillate branches bearing conidia emerging from moistened "calico-wood."

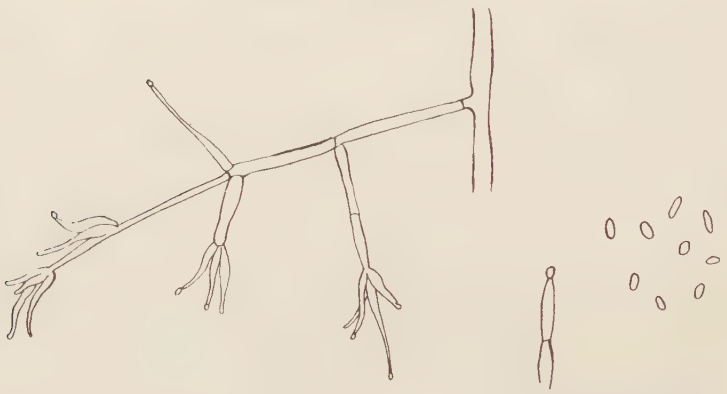


Fig. 13. Conidiophore and conidia of *D. concentrica* grown on bouillon gelatine.

This description agrees with those of *Daldinia concentrica* (Bolt.) Ces. et de Not. given by Tulasne in *Selecta fungorum Carpologie*, T. II, 1863; by Möller in "Phycomyceten und Ascomyceten," *Botanische Mittheil-*

ungen aus den Tropen, Schimper. Heft IX, p. 264, 1901; by Molliard, "Forme conidienne de *Daldinia concentrica*," *Bull. Soc. Myc. de France*, T. XX, pp. 55–60, 1904; by Bayliss Elliott in "On the formation of conidia and the growth of the stroma of *Daldinia concentrica*," *Trans. Brit. Myc. Soc.* vol. VI, pt III, 1920; and by others.

(2) The ash wood to which the ascocarps of *Daldinia concentrica* were attached showed to the naked eye brown streakings and black hyphae in the spring vessels. Under the microscope, the characteristic

Fig. 14.

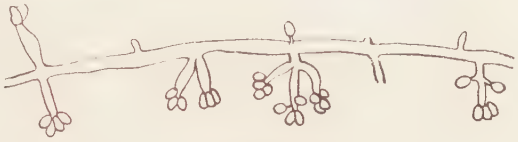


Fig. 15 (*a*).

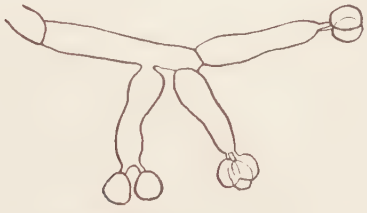


Fig. 15 (*b*).

Fig. 15 (*c*).

Fig. 14. Conidiophore and conidia produced on ash wood after artificial infection.

Fig. 15. (*a*) Conidiophore on malt agar, (*b*) part of same enlarged, (*c*) hypha in darkened malt agar turning brown at the tip owing to the change in colour of its contents.

dichotomously branched black hyphae and straight brown hyphae were observed in the wood and the bark, while colourless hyphae were present in nearly all the cells and vessels. Brown streaking was not so pronounced in this wood as it was in the first pieces examined, and occurred only in the bark and the adjacent wood.

(3) The ascocarps attached to the outside of this wood extruded ascospores in threads. When these fruit-bodies were kept in a damp atmosphere any ascospores adhering to them germinated and produced conidiophores and conidia identical with those emerging from the first piece of wood.

(4) The conidia used to infect the pieces of wood in the tubes germinated in all cases and produced mycelia. The mycelium was at first submerged in the water at the bottom of the tube or clung to the moist surface of the wood, but it soon began to penetrate the wood; and in a few weeks the blocks were covered on the outside with a mycelium bearing conidiophores and conidia, and were infected internally with hyphae. Colourless hyphae occurred in all the tissues of the wood, brown and black hyphae not being produced until later. Black dichotomous hyphae were produced on the outside of the wood before they were formed inside; and likewise, the liquid in the tube and the external tissues of the wood were turned brown long before any brown streaking could be seen inside the wood.

(5) Ascospores germinated and infected the wood blocks in precisely the same way, the mycelium and the brown colour produced agreeing in all cases with those produced by the conidia.

(6) Growth of the mycelium derived from the germination of conidia on agar and gelatine media took place on each of the different kinds mentioned before. On sucrose, malt, turnip, potato, prune and starch agar, *i.e.* carbohydrate agars, development was similar, resulting in all cases in a mycelium bearing conidia and black dichotomous hyphae, and the blackening of the medium. On plain agar, bouillon and peptone gelatine, no black or brown colour was formed either in the medium or in the hyphae.

(7) The results of the growth of ascospores on agar and gelatine media were the same as those obtained for conidia.

(8) In all of the liquid media mentioned, both conidia and ascospores germinated and produced larger or smaller mycelia.

In water to which no food material had been added, both kinds of spores germinated and even produced small mycelia probably at the expense of the ungerminated spores. The hyphae so produced were colourless, shortly and irregularly segmented, while the ends of some of the hyphae bore verticillate branches with conidia, though these feeble conidiophores might be replaced by clumps of branches segmented into oidia: no aerial hyphae were formed (Figs. 16 and 17).

In malt solutions the submerged mycelium was similar to that just described, but the segments were longer: there was no sign of production of conidiophores, but the conidia were budded off from any submerged hypha and these spores could in turn bud like a yeast. When the mycelium was so large that part reached the surface of the liquid, a typical aerial mycelium was formed, with pink conidial hummocks,

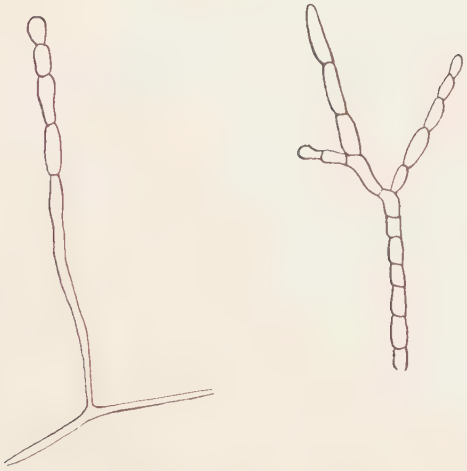
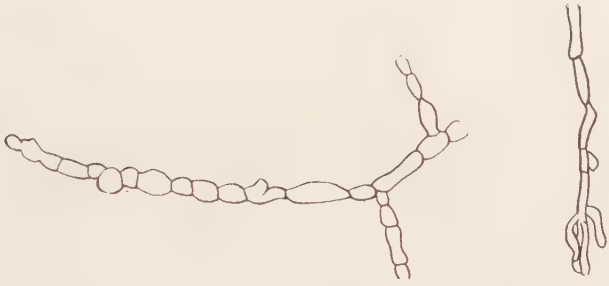
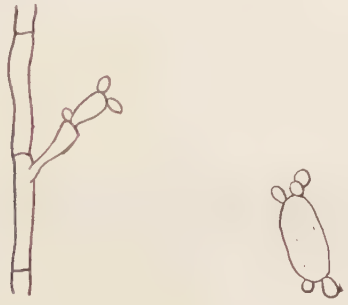


Fig. 16. Oidial form of hyphae submerged in water.



(*a*) (*b*)

Fig. 17. (*a*) Short-segmented hypha, and (*b*) conidiophore, submerged in water.



(*a*) (*b*)

Fig. 18. (*a*) Hypha bearing spores, and (*b*) budding spore, submerged in malt solution.

conidiophores and black dichotomous hyphae. The hyphae just below the surface and the top part of the malt solution became dark brown in colour (Fig. 18).

In sucrose solutions there was still further differentiation of hyphae. After several months growth, the submerged mycelium and the liquid were dark brown; and the hyphae were of three kinds: (1) very dark brown to black, wide, short-segmented and sometimes monstrous in width; (2) extremely fine, colourless or with patchy brown contents, and long-segmented; (3) intermediate between (1) and (2) and often including segments swollen into vesicles (Fig. 19).

Other solutions. Growth was fair in beech hemicellulose and onion pectin solutions but was extremely feeble in Citrus pectin solution. The mycelium developed well in all the other solutions, and a brown colour was produced in all except inulin, mannite, Citrus and onion pectin, and beech hemicellulose.

Conclusion. These cultures prove that one species of fungus, *Daldinia concentrica*, alone produced the symptoms in the original sample of "calico-wood."

Development of fungus.

On carbohydrate agar, the *conidium* germinated in a day or two by producing a germ-tube which emerged at one end or from the side and branched soon, or not until after the formation of the first septum. Branching and growth continued until a circular mat of creeping, colour-

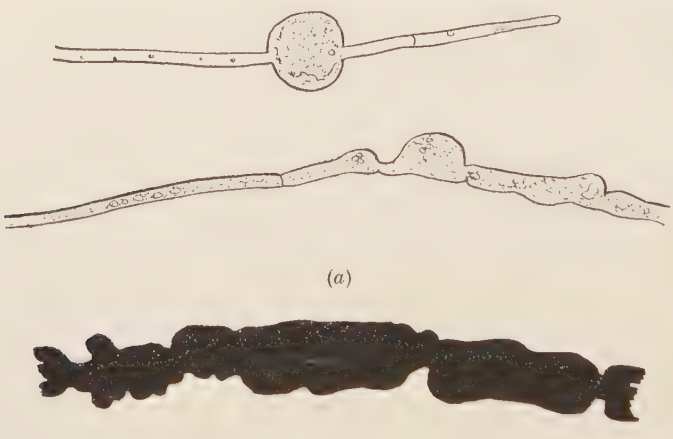


Fig. 19. (*a*) Hyphae bearing vesicles, (*b*) monstrous black hypha, submerged in sucrose solution.

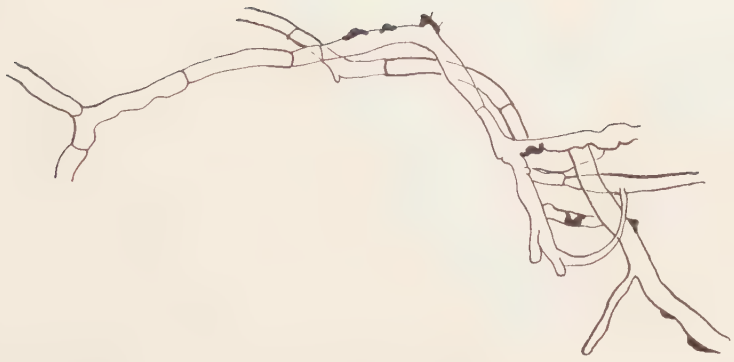


Fig. 20. First stage in development of black dichotomous mycelium—brown pigment beginning to be deposited, outside colourless hyphae, in small drops.

Fig. 21. Fig. 22.

Fig. 21. Later stage, increasing amount of external black pigment.
Fig. 22. Dichotomous branches from colourless hypha.

less, septate hyphae was formed. A week after sowing, the mycelium was white and fluffy, due to the presence of aerial hyphae which eventually became the conidiophores. After the mycelium was well-grown, darkening of the agar took place; this started in the centre, *i.e.* under the oldest part of the mycelium, and spread outwards until all the agar was changed from normal yellow to dark brown or black. This darkening of the agar seems to correspond to the brown streaking in the ash wood.

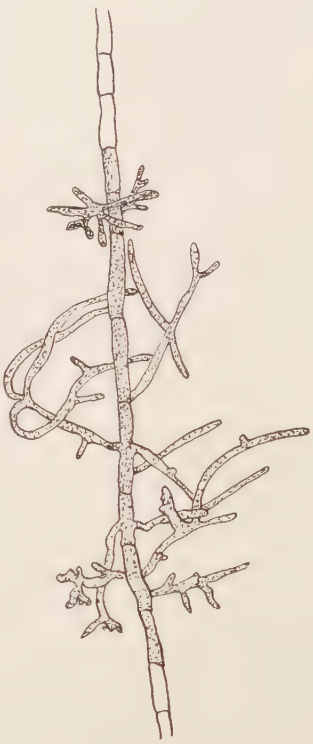


Fig. 23. Brown dichotomous hyphae branching off an originally colourless hypha.

At the same time new types of mycelia were produced. These were minute, spherical, black bodies, about 300 μ in diameter, consisting of matted dichotomous hyphae identical with those described as occurring in the wood vessels. The origin of these bodies was as follows: colourless branches arose from the original creeping hyphae, which forked dichotomously; a brown pigment was deposited outside the branches and held in oil-like drops on the hyphae. This pigment either at once diffused uniformly throughout the cell-wall of the hyphae or remained outside in droplets for some time. The branches increased in number, continuing to branch dichotomously, until the mature body was formed, in which the hyphae were black and without contents and often had black drops of pigment outside. No colourless hyphae with contents, nor spores, were associated with these bodies, so that they could not have been sclerotia. These bodies arose first in the centre of the mycelium and later on others arose nearer to the periphery; they were aerial, or partly or wholly embedded in the agar (Figs. 20–23).

Ordinary creeping hyphae embedded in the blackened agar became brown owing to the change in colour of their contents.

A sickly sweet smell was always produced by the fungus on agar medium.

The *ascospore* germinated, as figured by Molliard (6), by splitting of the outer layer of the wall down one side; the brown outer membrane was pushed aside by a hyaline swelling which gave rise to the germ-tube

which branched very soon to produce the creeping hyphae. Subsequently the typical conidiophores and black dichotomous mycelia were formed and the agar medium was turned dark brown.

The significance of the black dichotomous mycelia is unknown, although they are produced so abundantly. Molliard(6) describes how they are produced when the mycelium growing on carrot in a test-tube comes in contact with the glass, which suggests that they are due to desiccation. This can hardly be the case since they are produced also in liquid media and embedded in agar media. They might be compared with the brown hyphae temporarily formed by *Armillaria mellea* (*Agaricus melleus*) described by Hartig(3) in the wood of its host, since both kinds lose their protoplasmic contents, while those of *Armillaria* also lose their walls and are dissolved by the tree; and both might possibly function as temporary food reservoirs or excretory organs. Another suggestion is that the black mycelium represents an arrested development of ascocarps, and is therefore comparable with the sterile yellow mycelium (representing an arrested basidiocarp) of *Lenzites* outside wood.

External conditions of growth.

1. *Water supply.*

The following facts suggest that this fungus demands a considerable amount of water in the wood:

(1) *Daldinia concentrica* is one of the ascomycetes, the wood-attacking members of which generally need a considerable supply of water in the wood.

(2) The vegetative mycelium grows exclusively inside the wood, only the conidiophores and ascocarps being produced externally.

(3) A submerged mycelium can be formed and live quite well in various liquids.

(4) When a rod of ash wood was placed in a plugged test-tube with water at the bottom, and infected with either the spores or mycelium of the fungus, there were most hyphae in the wet wood above the liquid, the amount decreasing downwards in the totally submerged wood and upwards in the drier wood. This demand for water harmonizes with the fact that the fungus grows in the sapwood of the ash.

2. *Oxygen.*

The fact that the mycelium can grow submerged in aqueous solutions suggests that a feeble supply of oxygen suffices at least for the development of the vegetative mycelium. Attempts were made to cause the spores to germinate in anaerobic conditions, *i.e.* in sealed tubes of aqueous malt solution or malt agar, but with no result, so apparently a certain amount of oxygen is necessary for germination. No experiments were conducted on the resistance to carbon dioxide.

3. *Alkalinity and acidity of the medium.*

As both ascospores and conidia were able to attack and penetrate sound wood, it was surmised that they did not need for germination an acid medium. The results of cultures where the spores were placed in sucrose solutions containing different percentages of citric acid, acetic acid and caustic potash are given in Table I, the presence of *a* in a column of percentages indicating that *ascospores* were able to germinate in that percentage, of *c* that *conidia* germinated; both kinds of spores were tried in all the percentages mentioned, but they germinated only in those marked.

Table I.

Acid							Neutral	
·12	·10	·08	·06	·04	·02	·01		
—	—	—	—	—	—	*c*	*c*	Citric acid
—	—	—	—	—	*a*	*a*	*a*	
—	—	—	*c*	*c*	*c*	*c*	*c*	Acetic acid
—	*a*	*a*	*a*	*a*	*a*	*a*	*a*	
—	—	—	—	—	—	—	*c*	Caustic potash
—	—	—	—	—	—	—	*a*	

Alkali													
·02	·04	·06	·08	·10	·12	·14	·16	·18	·20	·22	·24	·25	·26	
—	—	—	—	—	—	—	—	—	—	—	—	—	—	Citric
—	—	—	—	—	—	—	—	—	—	—	—	—	—	acid
—	—	—	—	—	—	—	—	—	—	—	—	—	—	Acetic
—	—	—	—	—	—	—	—	—	—	—	—	—	—	acid
c	*c*	*c*	*c*	*c*	*c*	—	—	—	—	—	—	—	—	Caustic
a	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	*a*	—	potash

Both ascospores and conidia germinated in greater concentrations of alkalinity than of acidity, and in the case of the two acids and the alkali the ascospores withstood twice the strength that was toxic to the conidia.

These results contrast well with those given by Möller (5) for the germination of spores of *Merulius lacrymans* (*domesticus*). Dealing with

inorganic acids in distilled water, he obtained germination in 0·001 per cent. sulphuric acid, in 6 per cent. phosphoric acid (commercial acid, 25 per cent. H_3PO_4) and in 0·25 per cent. commercial hydrochloric acid plus 0·06 per cent. malt. Using organic acids, the optimum percentage for germination was 5 per cent. in the case of malic, citric and tartaric acids, 1 per cent. for oxalic acid, and with lactic acid germination took place in concentrations ranging from 10 per cent. to 0·001 per cent. He also attempted to germinate the spores in 10 per cent. to 0·0005 per cent. potash, but with no result. Thus the *optimum* for germination of *Merulius* in the case of citric acid was 5 per cent., but the *maximum* for *Daldinia* was below 0·04 per cent.; and whereas the spores of *Merulius* will not germinate in a solution of caustic potash containing 0·0005 per cent., the ascospores of *Daldinia* will do so in a solution of 0·25 per cent., the conidia in one of 0·12 per cent. Thus the spores of *Merulius lacrymans* need an acid medium and do not attack sound wood in the absence of an acid, while the spores of *Daldinia*, which lives either as a saprophyte or as a parasite on *Fraxinus* or *Alnus*, prefer a neutral medium, and can attack sound wood.

FOOD MATERIAL OF FUNGUS.

As the microchemical reactions of the lignified walls gave no clue to the food material used by the fungus, cultures were made on various nutrient media to determine what food materials it was capable of utilising.

At the same time observations were made on the production or non-production of the brown substance.

1. *Progress of mycelium on nutrient media.*

Various kinds of wood were used on which to grow the fungus, and included the sapwood of sycamore, oak, hornbeam and ash; the heartwood of mulberry, pine, larch, elm, walnut and American birch; and the ripewood of beech. The fact that the fungus lived on these heartwoods proves that it must be independent of starch, sugar and protein. At the same time, its power of dealing with the wood walls as regards the main structure is feeble, as shown by the firmness of the wood, passage of hyphae solely through the pits, and no change in lignin or pectin reactions.

Proof that the fungus can deal with starch is given by the wood cultures and by starch media. In the rod of ash wood infected with fungus in a test-tube, already described, both the relative amounts of

hyphae and of starch were determined, and it was clear that where there were most hyphae there was least starch. Moreover, after the fungus had been growing on starch agar and also soluble starch in water for some time, these were tested, for starch (with iodine) and for sugar (Fehling's solution), but both were absent.

That the fungus could grow in and utilise as food other substances is shown in Table II, which records the growth and presence or absence of the brown substance in media containing different carbohydrates[1].

Cotton wool with the addition of inorganic salts supported the fungus; the cotton eventually became brown and the strands decomposed or eaten away in places.

Table II.

Substance used by fungus as food	Growth	Colour
Solution in water:		
(1) Sugars		
Pentoses		
Arabinose 2 %	Fair	Pale brown
Xylose 2 %	Moderate	Pale brown
Hexoses		
Dextrose 3 %	Fair	Brown
Levulose 2 %	Good	Brown
Galactose 2 %	Good	Brown
Disaccharides		
Sucrose 2–20 %	Good	Brown
Maltose 5 %	Good	Brown
(2) Polysaccharides		
Starches		
Potato starch 1–5 %	Good	Brown
Inulin 3 %	Good	○
Lichenin 0·5 %	Good	Brown
Pentosanes		
Beech hemicellulose B 0·5 %	Moderate	○
Pectic bodies		
Onion pectin 0·5 %	Good	○
Citrus pectin 0·5 %	Feeble	○
Compound substances:		
Agar, plain	Poor	○
Agar, carbohydrate 2–20 %	Good	Brown-black
Agar, peptone 2 %	Moderate	Pale brown
Gelatine, peptone	Fair	○
Gelatine, bouillon	Fair	○
Wood, Ash	Good	Brown
Cotton wool + solution of 1 % KNO_3, 0·5 % KH_2PO_4, 0·2 % $MgSO_4$	Good	Brown

[1] The presence of a ○ in the table indicates the absence of the brown colouring matter.

2. *Nature and production of brown substance.*

The brown substance produced both in wood and in synthetic media, solid and liquid, was insoluble in the following reagents: acetone, alcohol, benzol, petrol ether, concentrated sulphuric, hydrochloric and nitric acids, and strong potassium, ammonium and sodium hydroxides whether used hot or cold. The only positive reaction that was obtained, was a change in colour from brown to bottle-green in the presence of any alkali, strong or weak, and a very slight change to reddish-brown with acids. These changes were reversible, for if the material were neutralised again, the original brown colour returned. The tests were made on the streaked wood, brown agar and brown liquid culture media, hyphae with brown contents and brown dichotomous hyphae, with the same results in each except in the case of the brown hyphae, which only gave a positive reaction when young (on agar) and not when old, as *e.g.* inside wood or constituting a fruit-body.

With regard to the origin of the brown substance, it seems likely that it is formed directly or indirectly from the decomposition of carbohydrates. From the table above it can be seen that pentoses, hexoses, disaccharides and polysaccharides all yielded a brown colour. Although the fungus grew in water or on plain agar and proteins, no brown colour was produced, neither was it produced in inulin, pectic bodies nor the pentosane beech hemicellulose. The cultures showed that the amount of coloured substance produced was not proportional to the vigour of growth, but was determined by the composition of the nutrient medium. It therefore seems probable that in the sapwood of ash, either the starch or sugar in the cell-contents or some carbohydrate in the cell-walls is decomposed, and the brown colouring matter so formed stains the walls and the contents of the lumina, or that both these processes take place with the same result. In one case it was seen that the brown colour was confined to the middle lamella in immediate continuity with the pit-closing membrane. Moreover in a number of cases, the colouring body present was confined to the cavities of the pits. These facts suggest that the attack on the wall takes place first, *via* the pits, on the middle lamella immediately surrounding the pit-membrane. There are a number of cases known in which, according to Hartig(4), the middle lamella is particularly attacked, with the result that the wood-constituents become isolated (*e.g. Pinus sylvestris* and *Trametes pini*, *Quercus* and *Thelephora perdix* (*Stereum frustulosum*)).

Again it is possible that this brown substance is allied to humus.

Wehmer(8) in a paper "Lignin and Humin substances in the fungous decay of wood" draws attention to the fact that a substance rich in carbon is produced in wood attacked by *Merulius lacrymans*, and that the same substances are extracted from *Merulius* wood as from peat, suggesting that lignin in wood may be decomposed to form humin, a fact which Schorger(7) substantiates when dealing with the fermentation of wood by filamentous fungi.

Summary and conclusion.

(1) Calico-wood (ash wood) was found to be infected with hyphae of two kinds: (*a*) black, dichotomously branched, and (*b*) colourless. In certain patches, the wood walls and cell-contents were stained brown.

(2) Conidia, obtained by keeping some of the wood moist, and ascospores, taken from the ascocarps of *Daldinia concentrica*, were grown on various solid and liquid media. The mycelia and the discoloration of the medium resulting from the germination of the conidium agreed with those resulting from the germination of the ascospore and with the hyphae and brown staining in the calico-wood.

(3) The morphology and development of the mycelium in the wood, the results of the growth in culture of the conidia and ascospores, and the comparison between calico-wood and ash wood attacked by *Daldinia concentrica*, proved that the fungus in calico-wood was *D. concentrica*.

(4) The external conditions of growth as regards water, oxygen and alkalinity and acidity, the food materials utilised by the fungus inside the wood, and the nature and production of the brown substance were investigated. Certain facts suggest that the attack in the wood begins in the middle lamella in contact with the pits.

(5) Calico-wood is therefore the wood of *Fraxinus excelsior* infected with *Daldinia concentrica*.

My thanks are due to Prof. Percy Groom, F.R.S., for his suggestions and helpful criticism, and to the Forestry Commissioners for their assistance in securing specimens of ash from the standing tree attacked by *Daldinia concentrica*.

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ADDITIONAL HOSTS OF *SYNCHYTRIUM ENDOBIOTICUM* (SCHILB.) PERC.

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(With Plates XVIII and XIX.)

Introduction.

In connection with the investigations on Wart Disease of Potato being carried out in the Rothamsted Experimental Station it was found necessary to test the infectivity of *Synchytrium endobioticum* on a number of additional species of plants. In selecting plants for this experiment, it was considered advisable to include not only such species of the Solanaceae as occur in the British flora and in cultivation in this country, but also certain plants which are weeds and crops of other countries in which *Synchytrium endobioticum* has been recorded.

Four species of American weeds were selected, *Solanum Commersonii* and *Solanum Jamesii* to represent the tuber bearing class; *Solanum nodiflorum* and *Nicandra physalodes* among the non-tuber bearing weeds. With the exception of *Solanum nodiflorum*, these species have been tested in America and *Solanum Commersonii* (7) and *Solanum Jamesii* (7) found susceptible to wart disease, while infection has not been recorded in *Nicandra physalodes*.

Among the British solanaceous weeds *Solanum nigrum*, *Solanum dulcamara*—including a white variety *Solanum dulcamara alba*—*Datura stramonium* and *Hyoscyamus niger* were chosen. Of these *Solanum nigrum* (2), *Solanum dulcamara* (2) and *Hyoscyamus niger* (3) have previously been found susceptible and were included rather in the nature of controls.

Solanum dulcamara var. *villosissimum* and *Solanum villosum* are common continental weeds. *Solanum villosum* is occasionally found in this country on waste ground in the neighbourhood of docks. *Petunia violacea*, *Petunia alba*, *Salpiglossis sinuata*, *Nicotiana affinis* and *Nicotiana Sanderae* are members of the Solanaceae which are commonly met in garden cultivation in this country. As examples of crop plants tobacco, tomato, egg plant and winter cherry were chosen. Of the above infection has been recorded in tomato (7) and suspected in Salpiglossis.

CULTIVATION OF EXPERIMENTAL PLANTS.

The plants were all raised and grown in a warm glasshouse throughout the experiments. The seed was sown in clean soil, and as soon as the young plants were large enough to handle, they were transplanted into pots of seven inches diameter. The compost was made of fresh potting soil well mixed with one half its bulk of soil contaminated with *Synchytrium endobioticum*. Previous workers (4, 5), investigating the conditions for infection of the potato by Synchytrium, have found that a certain period of excessive moisture in the soil results in a greater percentage of infection. In order to maintain these conditions, the pots were not crocked in the usual manner but cavings or sphagnum moss was used instead, so that there might be a free upward passage of water from the saucers in which the pots stood. The seedlings were treated normally until they were well established, *i.e.* from seven to fourteen days after transplanting, then earthenware saucers were placed under the pots and the amount of water supplied was increased so that some water always remained in the saucers.

Although the plants were grown under these conditions of practically water-logged soil until the experiments were concluded they grew well, with very few exceptions, and looked particularly healthy—flowering and fruiting as satisfactorily as under more normal conditions.

METHODS OF INOCULATION.

(*a*) *Soil inoculation.*

Decaying potato warts were finely ground and mixed with the compost. All the plants were grown in this contaminated soil which was kept very moist to give the most suitable conditions for the germination of the contained winter sporangia. In susceptible potatoes rapid and profuse infection is usually obtained in the collar region. A small amount of very highly contaminated soil (ground wart mixed with a little sand) was therefore placed around the collars of the experimental plants to increase the chance of infection in that area.

(*b*) *Glynne's "green wart" method.*

This method (5) depends on the more rapid and certain germination of the summer sporangia in a green wart in comparison with the resting sporangia contained in a black wart. The method consists of placing a piece of young and freshly formed wart in contact with the plant to be

inoculated. A film of water is maintained between the plant and the wart to ensure the correct conditions for infection, *i.e.* free water into which the zoospores from the summer sori may be discharged.

All the wart employed in these experiments was derived from Arran Chief potatoes. This variety is very susceptible to *Synchytrium endobioticum* and the warts are produced rapidly and in abundance on the young shoots. The warts were removed as soon as they were of a convenient size to handle, from 0·5 cm. in diameter upwards, and while of a pale yellow or green colour.

The actual procedure was as follows. A leaf axil on a plant was selected in which the young axillary shoot was just beginning to grow; about the third node from the ground level in most cases. A small wart or piece of wart was placed in contact with the young shoot and base of the leaf and then covered with moist sphagnum moss. The wart and moss were tied firmly in position with bast to ensure contact with the host plant. The sphagnum was moistened at regular intervals with water to keep the wart fresh and to preserve a film of water between the wart and young shoot.

In *Hyoscyamus niger* the wart was tied to one of the young leaves as the plants were in the sessile rosette form, not having produced a central stem.

The wart was left in contact with the shoot for varying periods and the plants were examined at intervals for signs of infection. The usual period of contact was three weeks, although certain plants showed warted outgrowths after fifteen days.

(*c*) *Attempted localisation of infection by swarm spores.*

This method is a modification of Glynne's method and was tested on Arran Chief potatoes. The aim was so to localise the point of inoculation that the manifestation of the disease might be confidently looked for in a small circumscribed area, thus saving much tedious examination by lens and eye over a comparatively large area of the shoot.

Tubers were selected with a shoot about 1 cm. long. The latter was preferably situated at the rose end and developing somewhat obliquely outwards from the long axis of the tuber. The other shoots were rubbed off. Small glass cups were made with a minute aperture near the base and so placed that the aperture came in contact with the shoot. Each cup was held in position against a young shoot by brass wire. A small piece of green wart was put in the cup and a drop of water added to

ensure contact between the wart and the small area of tissue exposed through the aperture in the bottom of the cup.

The tubers were placed in an incubator in wire cages and the wart frequently moistened. The shoots were examined at intervals for infection by Synchytrium but in no case was infection obtained. Although the method was not successful in these tests there seems reason to consider that some slight modification in the technique might make this an extremely useful method.

EXAMINATION OF PLANTS.

Infection from the soil.

The plants were removed from the pots and the roots and collar carefully washed free of soil. They were then thoroughly examined and any suspected portions removed and hand sectioned.

Infection by green wart method.

The young shoot and leaves of the plant which had been in contact with the wart were minutely examined and any portions bearing the slightest visible excrescences were removed and fixed in Flemming's weak solution. In some cases, even where no roughness or blistering could be discerned, samples were removed and fixed, in the hope of finding the parasite present in the tissues without any outward manifestation of its presence. The material was microtomed and permanent preparations made. The results of the experiments are given in Tables I–III.

RESULTS.

These experiments on the testing of Solanaceous plants for susceptibility to *Synchytrium endobioticum* have resulted in the finding of several new hosts for this fungus. Previous work on the infectivity of *Solanum nigrum*, *Solanum dulcamara* and *Lycopersicum esculentum* has been confirmed, and the following new hosts are here recorded: *Solanum dulcamara* var. *villosissimum*, *Nicandra physalodes*, *Solanum dulcamara alba*, *Solanum nodiflorum* and *Solanum villosum*. Infection has not been obtained in *Datura stramonium*, *Hyoscyamus niger*, *Nicotiana affinis*, *Nicotiana Sanderae*, *Nicotiana Tabacum*, *Petunia violacea*, *Petunia alba*, *Physalis Francheti*, *Salpiglossis sinuata*, *Solanum Commersonii*, *Solanum Jamesii* and *Solanum melongena esculentum*.

The reaction to the Synchytrium varied widely in the different hosts. On the majority of the species a definite warty outgrowth developed.

The production of a wart however was not taken as a criterion for infection. The presence of the parasite, in any stage in the tissues of the plant, has been accepted as evidence of a positive infection of the plant by Synchytrium, and the species so infected has been recorded as susceptible to the disease. This was considered the best attitude to adopt with regard to the very slight infections noted on some of the species, for example *Solanum dulcamara* var. *villosissimum*, *Nicandra physalodes* and *Solanum dulcamara alba*, because, although the infection was so slight, the parasite appeared to be perfectly healthy and capable of further development. The Synchytrium was never observed to be in process of disintegration after its entry into the host cell had been effected (1). If the host reaction or the amount of outgrowth developed is taken as an indication of the susceptibility of the host (6), then tomato, which produces warts easily, can be regarded as the most susceptible species examined. With this host a varietal test was conducted, the "green wart" method being employed for inoculation purposes, and of the fourteen varieties tested, all with the exception of three, Buckley, Sutton's Every Day and Sutton's Maincrop, proved susceptible to Synchytrium (see Table II). Kondine Red produced the most rapid reaction to the infection, the warted areas on the leaves being from 1·0–1·5 cm. in length within fourteen days of inoculation. Little pegs of tissue of about 0·5 mm. in diameter and 1–2 mm. in height were observed on the leaflets of the variety of "Fillbasket." These occurred either singly or in groups of two or three. Microscopic examination showed them to contain the fungus in various stages of growth. This form of host reaction is presumably an early stage before the distortion becomes more general over the leaf. In most cases the leaves of the plants of the successfully inoculated varieties were visibly distorted by the development of the fungus. The test confirms the fact that susceptibility to the disease is widespread among the varieties of tomato commonly used in cultivation in this country.

The Solanum group can be classed next to the tomato in degree of susceptibility or amount of host reaction. In this group warts were not produced with the frequency and regularity shown by the tomato. A definite warted outgrowth was produced on the leaf in one plant of *Solanum nigrum*, *Solanum nodiflorum* and *Solanum villosum* respectively. The remainder of the infections in these three species and in *Solanum dulcamara alba* were not easily visible as the hypertrophy of the leaf tissues had only just begun.

In the remaining hosts, *Solanum dulcamara* var. *villosissimum* and *Nicandra physalodes*, the infections were not visible to the naked eye.

The leaf of the former, which was found to contain Synchytrium in various stages, showed no visible external symptoms but in microtome section appeared to be slightly thicker than the normal leaf. There was none of the usual distortion which accompanies infection as in the tomato.

The infection in *Nicandra physalodes* was extremely slight. A young parasite was discovered near the median vein of a young leaf which was still folded, but its detection was quite impossible without microscopic examination. The pad of cells in which the parasite was situated is normal tissue covering the vein and not the beginning of hypertrophy of the host tissues.

The duration of these experiments was generally from fifteen to twenty-one days, and never more than five weeks, from the time the green wart was placed in contact with the shoot until the shoot was removed for examination. It is possible therefore, had it been practicable to leave the plants for a longer period, that the host reactions would have developed to a greater extent and that mature resting sporangia would have been found more abundantly in the tissues.

A varietal test was conducted with tobacco, six varieties being used (see Table III) and the green wart method employed as previously described. The results of the experiment were entirely negative.

The green wart test on *Solanum Commersonii* and *Solanum Jamesii* was attempted too late in the season, the haulms reaching maturity and withering before results could be expected. These plants will be tested again during the coming season and it is hoped to confirm Weiss's results.

In all experiments there was a total failure of any infection of the plants from the soil which indicates that for experimental purposes the green wart method of shoot inoculation is the more reliable. It is difficult to suggest any feasible explanation of the lack of infection from soil inoculation.

SUMMARY.

Infection of numerous species of Solanaceae by *Synchytrium endobioticum* has been obtained using Glynne's "green wart" method. Plants grown in contaminated soil did not show infection. The following new hosts are recorded: *Solanum dulcamara* var. *villosissimum*, *Nicandra physalodes*, *Solanum dulcamara alba*, *Solanum nodiflorum* and *Solanum villosum*.

In certain hosts the fungus may occur in the tissues with little or no external sign of its presence.

Table I.

Infectibility of different species of host plant.

Species	No. of plants inoculated	No. of plants infected	
		(1) Contaminated soil	(2) Green wart method
Datura stramonium	13	—	—
Hyoscyamus niger	4	—	—
Solanum dulcamara var. *villosissimum*	5	—	1
Lycopersicum esculentum	47	—	27
Nicandra physalodes	13	—	1
Nicotiana affinis	4	—	—
Nicotiana Sanderae	4	—	—
Nicotiana Tabacum	22	—	—
Petunia violacea	4	—	—
Petunia alba	4	—	—
Physalis Francheti	12	—	—
Physalis (Red fruit var.)	4	—	—
Salpiglossis sinuata	10	—	—
Solanum Commersonii	4	—	No test
Solanum dulcamara	2	—	1
Solanum dulcamara alba	4	—	1
Solanum Jamesii	4	—	No test
Solanum melongena esculentum	8	—	—
Solanum nigrum	4	—	2*
Solanum nodiflorum	8	—	4*
Solanum villosum	4	—	1*

* One visible wart.

Table II.

Tomata. Varietal test.

Variety	No. of plants inoculated. Green wart method	No of plants infected
A 1	3	1
Ailsa Craig	2	1
Best of All	3	3
Blaby	2	1
Buckley	2	—
Comet	2	1
Earliest of All	3	1
Fillbasket	2	1
Kondine Red	16	14
Manx Marvel	2	1
Princess of Wales	3	2
Sutton's Early Market	3	1
Sutton's Every Day	2	—
Sutton's Maincrop	2	—

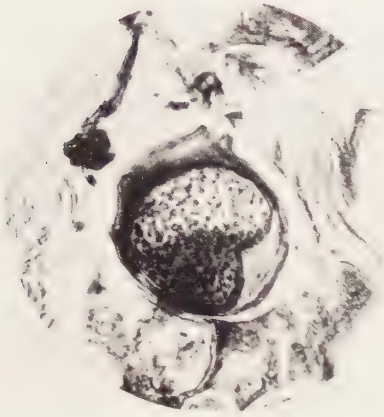


Fig. 1.

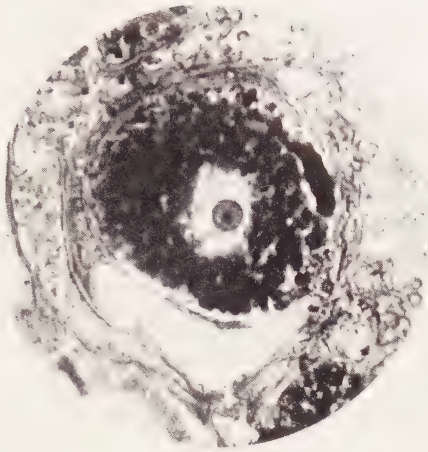


Fig. 2.

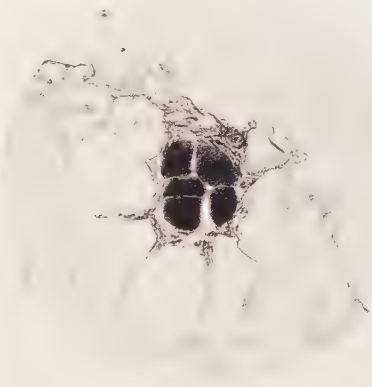


Fig. 3.

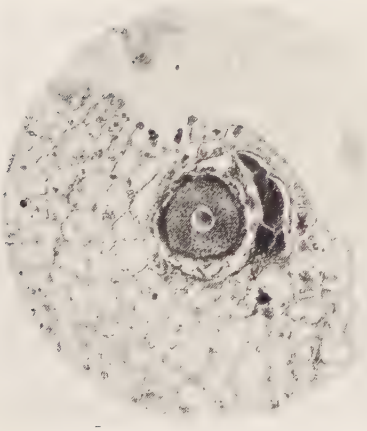


Fig. 4.

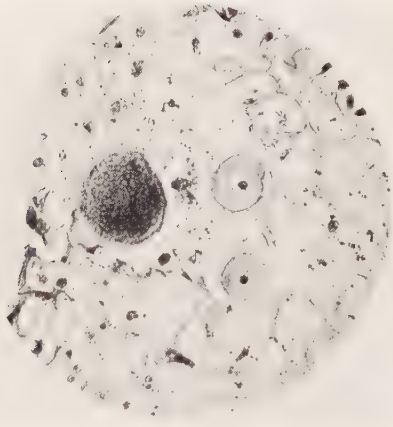


Fig. 5.

MARTIN.—Additional Hosts of *Synchytrium endobioticum* (Schilb.) Perc. (pp. 422–429).

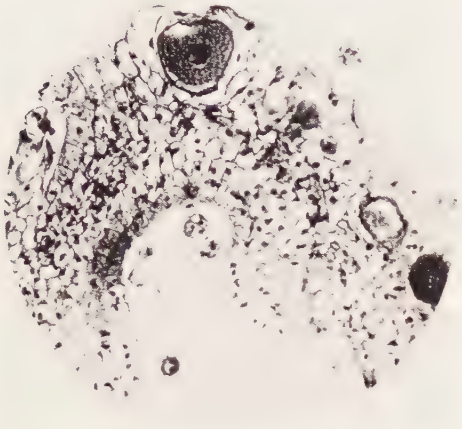


Fig. 1.

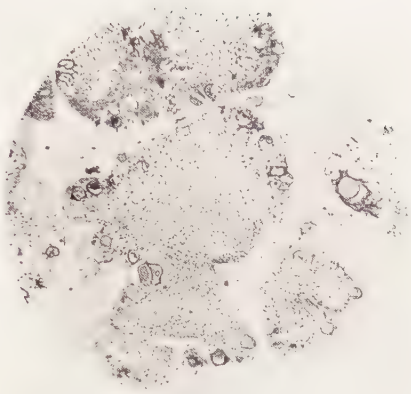


Fig. 2.

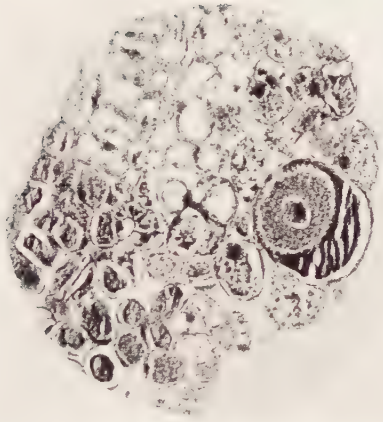
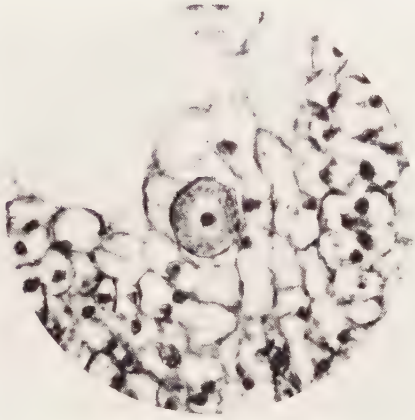


Fig. 3.



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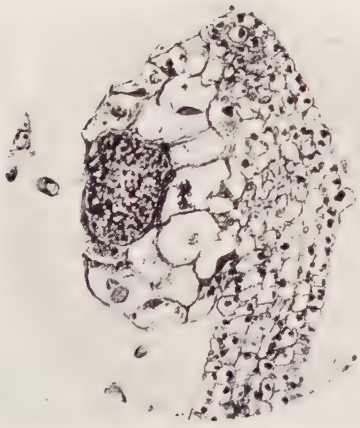

Fig. 5.

Fig. 6.

MARTIN.—Additional Hosts of *Synchytrium endobioticum* (Schilb.) Perc. (pp. 422–429).

Table III.

Tobacco. Varietal test.

Variety	No. of plants inoculated. Green wart method	No. of plants infected
Connecticut	3	—
Kentucky	3	—
Kentucky White Stem	3	—
Maryland	3	—
Virginia	3	—
White Burley	7	—

I am indebted to Dr W. B. Brierley for helpful criticism and suggestions received during the course of this work; and to the Director, Research and Experimental Station, Cheshunt, Herts, The Director, Royal Botanic Gardens, Kew, Surrey, The Regius Keeper, Royal Botanic Gardens, Edinburgh, and Martin H. Sutton, Esq., Erlegh Park, Whiteknights, Reading, for seed and tubers used in the experiments.

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EXPLANATION OF PLATES XVIII AND XIX.

PLATE XVIII.

Fig. 1. Winter sporangium of *Synchytrium endobioticum* in leaf tissue of *Solanum nigrum*.
Fig. 2. Young winter sporangium in leaf tissue of *Solanum dulcamara*.
Fig. 3. Five summer sporangia in a sorus in warted leaf of Tomato, variety Manx Marvel.
Fig. 4. Young winter sporangium in warted leaf of Tomato, variety Best of All.
Fig. 5. Young sporangia in warted leaf of Tomato, variety Kondine Red.

PLATE XIX.

Fig. 1. Sporangia in various stages of development in leaf tissues of *Solanum nodiflorum*.
Fig. 2. Warted leaf of *Solanum nodiflorum* showing sporangia in various stages of development.
Fig. 3. Sporangium in leaf tissues of *Solanum dulcamara* var. *villosissimum*.
Fig. 4. Young sporangium in leaf tissues of *Nicandra physalodes*.
Fig. 5. Young sporangium in leaf of *Solanum dulcamara alba*.
Fig. 6. Resting sporangia in warted leaf of *Solanum villosum*.

Addendum. Since going to Press infection has been obtained on *Solanum Jamesii*.

(*Received February 27th*, 1929.)

THE EFFECT OF COPPER SULPHATE ON TOMATO PLANTS

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Copper in various forms has by long use become an established constituent of many fungicides and is the basis of several proprietary preparations recommended for the control of some of the fungi which affect tomato plants. Some years ago Dr Bewley introduced the mixture known as Cheshunt Compound[1] as a control for "damping off" of tomato seedlings, a disease which, until then, had exacted considerable toll of most nurseries during the propagating periods. Unfortunately, the success of this preparation has tended to lead to its abuse and nurserymen in various parts of the country have used it so liberally as to render the possibility of copper poisoning a real one. Accordingly, the experiments recorded here were carried out to obtain information regarding the effects of copper on tomato plants at various stages of growth. The work was arranged to provide information regarding the effect of copper on:

(A) Germination.
(B) Young plants.
(C) Mature plants.

Experiments were also carried out to determine the effect of certain manures on the toxic effects due to copper in the soil.

Most of the experiments were repeated at different times in each of four years. Comparison of the results shows clearly that temperature, humidity and light intensity are factors which influence the results. An attempt to determine the significance of these individual factors was beyond the intention and scope of the present work and consequently the conclusions reached must be regarded as indicative rather than final. They are, however, likely to be of use to commercial growers.

[1] Cheshunt Compound consists of an intimate mixture of ammonium carbonate (11 parts) and copper sulphate (2 parts). The mixture is dissolved at the rate of 1 oz. in 2 gallons of water, and the solution watered on to the soil.

Effect of copper sulphate on the germination of tomato seeds.

In these experiments the soil was always air-dried before mixing with the salt and contained 2 or 3 per cent. of water. The salt was added either as powder and well mixed with the soil by hand or as a solution sprayed on to the soil, the whole being turned continually to ensure thorough mixing. After mixing the soil was damped to make it reasonably moist and allowed to stand for 24 hours before sowing. For the actual germination the ordinary seed boxes were used and 54 seeds sown in each box. The varieties Manx Marvel, Comet and E.S. 1 were used at different times and there appeared to be little difference in the results for the different varieties.

In Table I are shown *average* figures obtained in May 1927 for several batches of seeds. Seeds sown in October of the same year gave similar results, but germination was somewhat retarded throughout owing to the unfavourable weather conditions.

Table I.

Copper added as sulphate; % in soil	Percentage germination					
	5 days	6 days	11 days	15 days	18 days	22 days
0·0 (Controls)	15	20	30	100	100	100
0·1	50	55	55	80	80	80
0·3	65	95	95	95	95	95
0·5	25	45	45	70	70	70
0·75	15	25	35	45	45	45
1·0	0	0	0	0	20	20
2·0	0	0	0	0	45	45
3·0	0	0	0	0	0	0

Except in the controls the poison effects were apparent before the true leaves had developed. Even at the lowest concentration given in the table seedlings were very weakly and the cotyledons were of a dark colour. At the higher concentrations the seedlings made no growth and the root action was very restricted in all cases. The table suggests that the rate of germination is accelerated by the copper at a concentration up to 0·3 per cent. in the soil. The total germination, however, appears to be unaffected at this concentration.

A considerable amount of germination work was carried out between March and July 1928 in soils containing from 0 to 0·5 per cent. of copper. In all cases the inclusion of copper accelerated the rate of germination as already indicated in Table I. As before, however, the total germination

was unaffected and in all cases up to a copper content of 0·3 per cent., it occurred to the extent of 90–100 per cent. of the seed used in from 8 to 13 days, according to weather conditions. An attempt was made to determine the upper limit at which the maximum number of seed germinated. This was found to lie between 0·28 and 0·30 per cent. of copper. The figures in Table II are typical.

Table II.

March 1928		July 1928	
Copper added %	Percentage germination	Copper added %	Percentage germination
0·0	94	0·0	100
0·094	100	0·06	86
0·28	100	0·12	95
0·47	51	0·18	100
		0·24	95
		0·30	70

These results were duplicated several times and although the total germination may vary with conditions it is probable that above a concentration of 0·28 to 0·3 per cent. germination is definitely depressed.

That smaller concentrations have little permanent beneficial effect on germination is shown by the data in Table III, the results of which were obtained in July 1928.

Table III.

No.	Copper added %	Percentage germination		
		5 days	7 days	12 days
1	0·0	64	80	100
2	0·03	72	100	100
3	0·06	64	80	95
4	0·09	64	80	100
5	0·12	72	95	95
6	0·15	76	95	100

The figures in Table III suggest that in some cases germination has been stimulated. Unfortunately, however, the general effect on the seedlings is harmful. As soon as real growth is to be expected, the toxic effects of the copper manifest themselves. In general these consist of a darkening of the stems and cotyledons, and very pale leaves. An examination of the seedlings from the experiments quoted in Table III, 15 days after sowing, showed that there was little difference in Nos. 1, 2 and 3. The seedlings in No. 4 were dark in colour and not so healthy as the controls. Those in Nos. 5 and 6 were definitely stunted, the

cotyledons and stems being dark coloured and the young leaves pale. A few days later the seedlings in No. 2 were slightly taller than any of the others, but eventually none of those growing in the soil containing copper was as satisfactory as those in ordinary soil. In other words the stimulus to germination is more than nullified by the later toxic effects. Some of the seedlings from Nos. 1, 2 and 3 were potted up in ordinary soil and observations on them for the following three weeks revealed no differences. So the early benefit in germination is of no lasting value, even if the plants are potted up in new soil before the toxic effects of the copper appear.

Concurrently with these experiments with copper as sulphate some experiments were also made using Cheshunt Compound as source of copper. In all cases where any depressing effect was due to this mixture that effect was less marked than the effect due to the same concentration of copper added as the sulphate. The range of concentration over which Cheshunt Compound may be used is restricted as the ammonia in it is liable to scorch at concentrations very much above the normal strength, while even at low concentrations the ammonia is liable to stimulate the growth of young seedlings.

Experiments with young tomato plants.

The earlier experiments in this section were carried out in 1923 and 1924 and were arranged to compare the effects due to sulphate and those due to Cheshunt Compound. The compost consisted of a mixture of four parts of loam and one part of well rotted stable manure. During the period the light was never good, days were short and temperatures were too low for satisfactory growth. Under these conditions concentrations of the order of 0·2 per cent. of copper as sulphate caused the plants to be appreciably affected. When the copper was added as Cheshunt Compound, however, the plants were unaffected by considerably higher concentrations. Although growth was limited the plants affected by the copper showed the ill-effects within 3 days after planting. The foliage was exceptionally dark and some of the leaves were of a deep blue colour on the under sides. The roots were brown and apparently there was just sufficient activity to keep the plants alive but insufficient to enable growth to be made.

In the summer of 1924 attempts were made to determine the concentration of copper as Cheshunt Compound at which growth would be affected. If the mixture be used as directed by Bewley(1) the maximum amount applied to a young pot plant would not exceed 0·02 gm. of

copper, per pot of 300 to 350 gm. of soil. At this concentration no ill-effects have been observed even under conditions very adverse to the well-being of the plant. On different occasions young plants were treated with amounts of the mixture containing up to 0·55 gm. of copper per pot of soil. Here again the effect of environmental conditions was apparent but in no case was any effect noticeable with amounts up to 0·21 gm. of copper. Mention may be made of a typical series in which 300 gm. of soil were used for each plant. Batches of sturdy plants were potted and they received respectively 0, 0·069, 0·137, 0·206, 0·274, 0·343, 0·411 gm. of copper as Cheshunt Compound. At the end of three weeks all the plants had made appreciable growth. No difference could be discerned in the appearance of the plants in the first four batches but those in the last three were not so tall although showing no untoward symptoms. That is, up to a concentration of about 0·07 per cent. copper as Cheshunt Compound is innocuous.

The question next arose as to how much copper would be likely to be present in the soil round a young plant where Cheshunt Compound had been used repeatedly for some years. This is only a remote possibility on a large nursery since the same soil is not likely to require treatment in consecutive years, but it may occur on small nurseries where steam sterilisation is not practised. As ordinarily recommended 2 pints of the solution would be applied to the soil round each plant in any one year. In 10 years this would amount to 2·5 gallons and if it be assumed that *none* of this be washed out in the course of ordinary cultivation the accumulation would amount to 1·370 gm. of copper. Assuming that this is distributed round the plant in 10 lb. of soil (the soil content of a large pot) the concentration of copper is of the order of 0·035 per cent. As already shown in the previous paragraph this concentration is quite harmless to young tomato plants.

The effect of copper on mature tomato plants.

The effect of copper in both of the forms mentioned has been examined on plants ranging from large plants growing in 6-inch pots to plants in 12-inch pots and carrying fruit. In the main the results have confirmed findings already mentioned regarding the comparative effects of the two forms of copper, that is to say, copper as sulphate is always more potent than copper in the form of Cheshunt Compound.

Under conditions prevailing during the winter months the effects of copper in small concentrations are so slight as to be almost indistinguishable from the effects of the unfavourable conditions. During the

summer months the poison symptoms are easily distinguished and are visible within 2 or 3 days. As an example may be quoted the results obtained from well-established plants of an average height of 11 inches potted up during July 1928. The soil contained copper as the sulphate in amounts varying from 0 to 0·604 per cent. In 3 days all the plants in soil with 0·07 per cent. or more were dark in colour and at the maximum concentration used most of them were shrivelled up and apparently dead. Within 5 days the symptoms of the surviving plants were accentuated and at concentrations of 0·15 per cent. and over the plants were all in a very unhealthy condition. It is interesting to note that 15 days later some of these plants were still alive. By that time most of the lower leaves were dead, the tips were very pale and scorched, and the stems showed long brown streaks. At concentrations up to 0·07 per cent. however, the only effect was one of slightly reduced growth.

These results confirmed those found in 1923 and 1924. In this latter case however, tests were also made with the copper as Cheshunt Compound. Up to concentrations of 0·25 per cent. of copper in this form none of the effects associated with the sulphate was observed. Above this concentration no reliable information could be obtained since the ammonia introduced in the mixture always scorched the foliage. Comparisons made at other times confirm the conclusion that copper as Cheshunt Compound is less harmful than the same concentration of copper as sulphate.

To examine the effects on mature plants large pots containing 12 lb. of soil were used and the plants were grown to a height of 4 feet or so, so that four trusses of fruit developed on each plant. Batches of soil were made up to contain copper as sulphate at concentrations of 0, 0·02, 0·04, 0·08, 0·12, 0·16 and 0·19 per cent. respectively. The plants were manured as often as was necessary and the fruit was picked as it ripened. Unfortunately none of the plants grew really well, irrespective of treatment, but it is noteworthy that even in the soil containing a concentration of 0·19 per cent. growth was made and fruit developed. The crop produced with 0, 0·02 and 0·04 per cent. was practically the same for all the plants. At the higher concentrations there was more variation in individual plants but the average per plant compared quite favourably with the control plants. As a matter of interest the seed in the fruit was saved. The different treatments appeared to be without any definite effect on the number of seed per fruit or on the germination of the seed. The subsequent seedlings however were all unsatisfactory for further growth, where the original plants had been treated with copper.

Treatment of poisoned soil.

The grower's most important concern is the treatment of soil which may have become poisoned, and the detection of the symptoms, which are more or less similar to those produced by most poisons. In seedlings the stems become blue or violet in colour and the cotyledons a dark green. The rough leaves are dark green on making their appearance but soon become a pale yellow, and the veins show in bold relief. These symptoms are accompanied by stunted growth and sometimes by distortion.

In young pot plants severe poisoning is accompanied by shrivelling of the lower leaves, brown streaks on the stems, and the leaves at the tips become yellow on the upper surface and often blue or violet on the under side. These plants do not necessarily die, but little or no apparent growth is made and when the lower leaves have fallen off the plants consist of vertical stems each surmounted by a tuft of stunted leaves.

With large plants the effects are not so definite and even with comparatively large amounts of copper no striking differences are noticeable between treated and control plants.

Washing with water.

When the concentration of copper in the soil is low much benefit is derived from heavy watering. When the concentration is so high as to cause immediate (*i.e.* within 2 days) injury no amount of water appears to remove the copper completely. For instance, in one series of experiments, some pots of soil which was highly toxic were watered daily for three weeks so that water flowed freely through the drainage holes on each occasion. At the end of this time the leachings, after concentration, showed none of the usual qualitative tests for copper. On planting in this soil the symptoms did not appear immediately but were quite definite at the end of eight days.

The effect of lime.

Hydrated lime has been found to give satisfactory results when applied to poisoned soil. In mild cases of poisoning a light dressing at the soil surface is usually sufficient to encourage normal growth. With more severe cases it is necessary to mix the lime with the soil and water the whole heavily, and when the mixing is really thorough the amount of copper which is rendered innocuous is surprising.

The effect of manures.

Stable manure, sulphate of ammonia, sulphate of potash and basic slag have all been found of some benefit. None of these, however, is as effective as hydrated lime. Of the four, application of sulphate of ammonia followed by watering is the most satisfactory.

The effect of soiling.

On many occasions plants showing the typical symptoms have made good recovery when one or two inches of "clean" soil have been added round the stems. Even in seed boxes where there has been little room for the addition of such soil seedlings have made excellent recovery after this treatment. This confirms the view that copper, although causing the plant very severe injury, often does so without actually killing it, and that the plant can live for appreciable periods in the presence of a toxic concentration. When more favourable root conditions prevail, as when new soil is added, new roots are formed and new growth is made.

Summary and conclusions.

An examination has been made of the effects of copper on tomato plants at various stages of growth.

No benefit appears likely to accrue from the addition of copper sulphate to tomato soils. The rate of germination may be stimulated but the total germination is unaffected and the subsequent growth of the seedlings is not satisfactory.

The toxicity of copper is greater when used as sulphate than when added in the form of Cheshunt Compound.

Cheshunt Compound, introduced as an emergency measure for the control of "damping off" of tomato seedlings and foot rot, is harmless when used as originally directed.

When soil is poisoned the addition of new soil round the stems of plants and the application of hydrated lime are beneficial.

The addition of small amounts of copper sulphate to soil in which mature plants are grown is without effect on the number of seed per fruit and on the ability of the seed to germinate, but the subsequent seedlings are unsatisfactory from the commercial point of view.

This work was carried out at the suggestion of Dr W. F. Bewley, to whom the author tenders his best thanks.

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SOME PROPERTIES OF THE CELL-WALL OF COTTON-HAIRS

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(With 3 Text-figures.)

The structure of the cell-wall of the cotton-hair and its behaviour under the action of various reagents have been the subject of investigation by numerous workers. Denham (1) and Clegg and Harland (2) have drawn attention to its porosity, whilst Calvert and Summers (3), and Coward and Spencer (4), have shown that the dried hair absorbs caustic soda solution until it assumes the same diameter that it has as a living cell inside the boll.

The relation of this porosity of the cell-wall to nutrition has been discussed by the present writer elsewhere (5), and subsequent investigations of the swelling of the cell-wall, before collapse occurs, have revealed unsuspected features correlating the somewhat difficult problems of hair weight, diameter and intrinsic strength.

Method.

The apparatus used in these investigations was a Leitz microscope projection apparatus with three lens substage condenser and centring mount, oil immersion lens, four eye-piece, and reflecting prism adjusted to give a magnification of 1250. With critical illumination and working within a radius of three inches from the centre of the field of projection, diameter measurements of 300 cotton-hairs can be made in one hour with an accuracy of ± 2 per cent. without any strain or fatigue to the eyes.

1. Stability of the cell-wall.

Diameter measurements were made on a tuft of hairs from an unripe boll of Ashmuni cotton 35 days old, mounted in tap water, 5 per cent. salt solution, saturated brine, 5 per cent. formalin and after immersion in boiling water. The same tuft of hairs was used for the various treatments, and all observations were made at the centre of the hair length. This is

facilitated by taking a tuft of hairs (say 150) attached to a portion of the seed coat. They are straightened out by combing under water and when mounted on the slide, the piece of seed coat is used as a stop for one edge of the coverslip from which to measure the centre of the hairs. The results are given in Table I.

Table I.

Treatment	Mean diameter in 1/1250 mm.
Tap water	27·3
5 % salt solution	27·4
Saturated brine	27·3
5 % formalin, 1st day	27·6
5 % formalin, 2nd day	27·6
After boiling water	27·1
Dried and mounted in water ...	21·8
Swollen in 18 % NaOH ...	26·9

From these results it appears that the diameter of the hair is unaffected by salt solution, formalin or boiling water. It is therefore concluded that the diameter of the cotton-hair is not dependent on osmosis or the vital activity of the cell, and that collapse of the hair on maturation is entirely a physical process dependent on dehydration of the cell-wall.

In the above instance, the original diameter of the hair was almost restored by immersing the collapsed hair in 18 per cent. caustic soda, thus supporting the theory that the cuticle determines the diameter of both the living and the "swollen" hair.

Fig. 1 is a reproduction of a photograph of living hairs mounted in water. One of the hairs shows the effect of partial removal of the primary wall and cuticle by friction. It will be seen that removal of the primary wall results in an expansion of the hair-cell, from which it must be concluded that the cuticle of the living hair is under tension.

Fig. 1.

2. Comparison of different strains of cotton under the action of caustic soda solution.

Previous workers have maintained that the limiting action of the cuticle on cell diameter applies to all cottons alike. Although Summers's results show differences between fine and coarse cottons, he attributed them to experimental error. The present writer found much greater differences than those of Calvert and Summers, and such as to be unaccountable by error. This could be explained by the fact that their material was grown in England under artificial conditions, and only one sample could be described as a fine cotton.

By making the measurements in water and caustic soda solution on the same tuft of hairs at the same portion of their length, the possibility of sampling error is avoided and the results have an accuracy of ± 2 per cent.

The results obtained for 20 strains of cotton are given in Table II, arranged in order of "living" diameter.

Table II.

Sample	Diameter measurements in 1/1250 mm. In water	In 18 % NaOH solution	Percentage increase in diameter
1	19·1	21·8	14·0
2	19·6	22·8	16·2
3	20·6	23·3	13·2
4	21·0	23·8	13·3
5	21·1	24·8	17·6
6	21·7	24·2	11·5
7	22·4	25·1	12·0
8	22·8	24·6	7·9
9	23·0	24·5	6·5
10	23·1	24·7	7·0
11	23·4	25·4	8·5
12	23·7	25·3	6·7
13	25·2	27·0	7·1
14	26·0	24·8	4·6
15	26·2	26·6	1·5
16	27·3	26·9	1·4
17	28·2	28·3	0·3
18	28·2	27·6	2·1
19	32·1	32·3	0·6
20	33·5	32·6	2·8

The results in the third and fourth columns are plotted in a curve (Fig. 2,) which shows that the increase in diameter on swelling the cell-wall with caustic soda solution varies inversely as the diameter of

the uncollapsed hair; and that when the diameter exceeds 26/1250 mm., there is no increase in diameter on swelling the cell-wall, but sometimes an apparent decrease. This unexpected decrease in diameter of the coarser hairs may be accounted for by a change in shape of the cross-section from an ellipse to a circle (see Fig. 3).

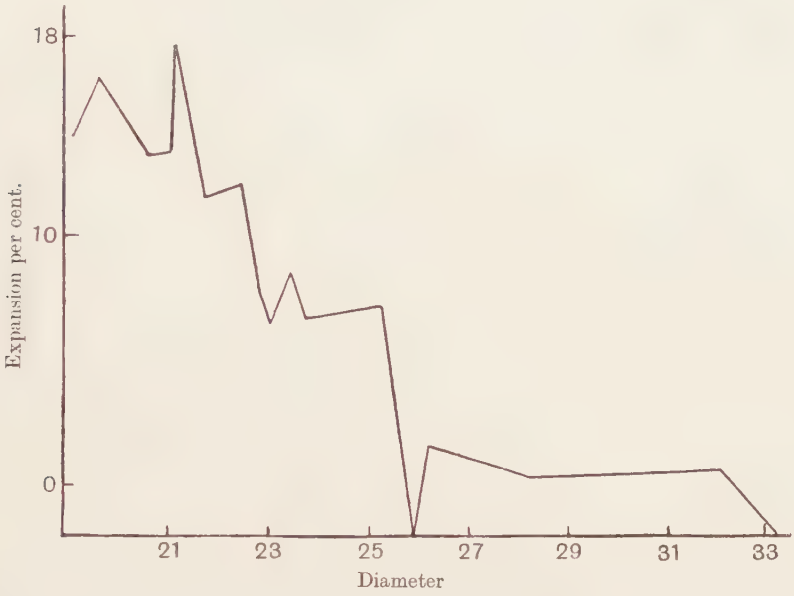

Fig. 2.

Fig. 3.

Thin-walled hairs of large diameter and a correspondingly large lumen would tend to assume an elliptical shape between the slide and coverslip, but, on swelling the cell-wall with caustic soda, the increased internal pressure would tend to "inflate" the hair into a more cylindrical tube.

This source of error in the measurement of the living hairs, due to the unavoidable presence of thin-walled hairs, would enter into all the

determinations irrespective of the variety of cotton, so that the expansions recorded for the finer cottons may be actually less than their true values. The varying amounts of this error would account for the irregularities in the correlation curve.

This increased expansion of the finer cottons is very remarkable, especially as the four cottons with the greatest expansion represent strains of Sakel remarkable for their superior spinning tests. No. 5, which gave the highest percentage expansion (17·6), possesses the highest intrinsic strength of any cotton grown in Egypt(6), whilst the four cottons at the bottom of the curve comprise one American, two coarse strains of Ashmuni and a sample of native brown cotton of no commercial value.

That there is some relation between original diameter and intrinsic strength is shown by the results in Table III for six different strains of cotton.

Table III.

Diameter of living hair in water	Intrinsic strength
19·1	283
21·0	303
22·4	260
22·8	267
28·2	223
33·5	207

Not only is this true for different strains of cotton, but it also holds good for cotton of the same strain. A sample of Sakel cotton was divided into bundles of hairs of different weights per cm., and the hair strength determined by means of the magazine hair tester.

The results are shown in Table IV.

Table IV.

No. of hairs in bundle	Hair weight per cm.	Hair strength	Intrinsic strength
75	13·3	4·0	300
81	13·7	4·23	308
76	17·0	4·43	260
83	17·2	4·7	273

It is reasonable to assume that, in cotton from the same plants, the hair weight per cm. is proportional to diameter. The above results, though few in number, are free from any sampling error, and show a definite relation between diameter and intrinsic strength.

Discussion.

The familiar "beading" of cotton hairs with a damaged cuticle on treatment with caustic soda is conclusive evidence that the cuticle determines the final diameter of the swollen cell-wall of all types of cotton.

To account for the increased diameter on swelling of fine cottons, we must suppose the cuticle to be more extensible than that of coarse cottons, and that it is probably subject to a greater pressure from the swollen cell-wall. The higher intrinsic strength of the cell-wall of fine cottons suggests a different molecular aggregation or polymerisation of the cellulose molecules, which would be associated with a greater absorption and swelling power in caustic soda solution.

The higher intrinsic strength of the finer hairs over the coarser hairs of the same plants is indeed remarkable, and suggests that the inherent properties of the cellulose (or degree of polymerisation) are in some way connected with the dimensions of the living hair. How this can occur is at present a subject only for speculation. The existence of internal pressure in the cell-wall of the living hairs, and the greater surface area for nutrition possessed by hair cells of small diameter, may well be the basis of all the complex factors determining quality in cotton.

Summary.

(1) The cell-wall of the living cotton-hair is under tension and its diameter is unaffected by osmotic pressure or the death of the cell.

(2) Increase of diameter of the uncollapsed hair by swelling in caustic soda varies inversely as the diameter of the hair in the case of fine cottons, and does not occur with coarse cottons.

(3) The diameter of the uncollapsed cotton-hair varies inversely as the intrinsic strength of the collapsed hair.

I have to thank Dr R. H. Pickard, F.R.S., and his colleagues Miss Calvert, M.Sc., and Miss Clegg, M.Sc., of the Shirley Institute, Manchester, for their very helpful criticisms.

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THE PRODUCTION OF ETHYL ALCOHOL AND ACETALDEHYDE BY APPLES IN RELATION TO THE INJURIES OCCURRING IN STORAGE

PART I. INJURIES TO APPLES OCCURRING IN THE ABSENCE OF OXYGEN AND IN CERTAIN MIXTURES OF CARBON DIOXIDE AND OXYGEN

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(With Plate XX.)

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Introduction.

In recent years problems of apple storage and the transport of apples from overseas have been engaging the attention of many workers in this and other countries. Some of the work is directed at providing stores in which home-grown apples may be kept in a marketable state for longer periods than in the past. Other work is proceeding on the conditions which prevail in the holds of ships carrying apples for long

distances, and often, indeed, across the equator in the summer. In general it may be said that the aim of all the work is to arrange storage conditions so that the risk of fungal and bacterial rot is minimised, and so that the inherent processes of senescence of apples can be retarded, or accelerated, at will.

The storage of home-grown apples and of apples in ships' holds in freely circulating air at low temperatures has already proved its worth, although, as we shall now see, it is not wholly without danger. In order to prevent freezing injury, the temperature should never be allowed to fall below 0° C., but even at temperatures ranging from 1° C. to 5° C. it has been found that certain varieties of apples may be injured more quickly than when they are stored at higher temperatures, and that these injuries are not brought about by micro-organisms. Names, such as "deep- or soft-scald," and "internal-breakdown," have been given to those physiological diseases which seem to be more often encountered at low temperatures (2, 4). These diseases will be considered in a later paper.

The storage of fruit in atmospheres other than air has often been considered. In gas storage trials carried out by Kidd and West (5) certain varieties of apples have been stored in an atmosphere of approximately 10 per cent. carbon dioxide + 10 per cent. oxygen + 80 per cent. nitrogen. The increase in the concentration of carbon dioxide in conjunction with the decrease in that of oxygen retards metabolic activity and so delays the ripening of the fruit. These workers have found that a temperature of 8–10° C. gives the most satisfactory results. No injurious effects have been noticed under these abnormal conditions so long as the composition of the gas mixture was rigidly controlled. If however, as a result of the respiration of the apples, the concentration of carbon dioxide increased even to 13–14 per cent., then, as Kidd and West have conclusively shown (3), a physiological disease incident in the interior, which they have called brown-heart, was liable to occur, provided that oxygen was still present.

Such harmful gas conditions as those described in the last paragraph may arise from the respiration of apples in air stores which are insufficiently ventilated, as indeed has happened in certain types of ships' holds carrying fruit, and, as a result, brown-heart has occurred on a large scale (3). Further, if a gas store or an unventilated air store is left without control, much if not all of the oxygen may be used up by the respiration of the apples which are then, as has long been known, sooner or later killed.

It has been recognised for some years that although the flesh tissue of apples appears to the eye to be homogeneous, it may behave as a heterogeneous population of cells when stored under adverse conditions, in that usually the cells are not all injured simultaneously. Indeed, up to the present, the physiological diseases of apples have been distinguished from one another mainly by the different incidence of injuries and the variations shown in their subsequent extension[1]. These differences and variations are implicit in the definite names, such as superficial-scald, deep- or soft-scald, internal-breakdown, brown-heart, which have been given to these diseases. Although these terms have proved very useful, they are not wholly satisfactory as they do not even adumbrate the causes of the diseases. Moreover, the same injurious effects may be produced under quite different storage conditions: for example, injuries incident in the epidermis and progressing inwards, often spoken of as deep- or soft-scald, have been reported as occurring in air stores at low temperatures (2, 3), in anaerobic stores at all temperatures (2, 3), and in stores containing certain mixtures of carbon dioxide and oxygen (1, 6). It is thought that it would be preferable in describing physiological diseases to use terms which both indicate the nature of the browning manifestations, and suggest some distinctive internal physical or chemical circumstance which either causes or can be correlated with the disorganisation of cells which always precedes browning.

A great deal of difficult work must be done before such a nomenclature can come into being, as a wide knowledge of the biophysical and biochemical changes, which occur under different conditions of storage, would be required. With this knowledge it is considered that it might be possible, after diagnosing a physiological disease by physical or chemical tests and by its appearance, to say what environmental conditions had caused the disease. Further, the chance of preventing physiological diseases will probably be greater when the internal phenomena associated with them are more fully understood. It was with a view to investigating the biochemical phenomena associated with these diseases that the author commenced this work under the Food Investigation Board in 1922. The experiments to be discussed under the title of this paper were planned to determine whether the injuries occurring in storage were preceded, accompanied or followed by zymasis. During this metabolic disorder the concentration of ethyl alcohol and acetalde-

[1] It must be remembered that the apple is composed of oxidase tissue, so that injury is always accompanied by browning if oxygen is present to complete the catechol-oxidase system (7).

hyde in cells progressively increases from the small amounts of these substances—ethyl alcohol 0·006 per cent.[1], acetaldehyde 0·0005 per cent.[1]—found in freshly gathered apples. The author has, in an earlier paper (9), described the methods of analysis which he uses in following zymasis. In this communication the injuries occurring in apples in certain gas atmospheres, other than air, will be considered.

In most of the work Newton Wonder apples were used, but similar experiments on other apples have been made, and so far have given results in general agreement with those which will now be described. It is realised, however, that many differences in detail may be found in further work on other varieties, and that a given variety may be found to behave differently according to its physiological state.

SECTION 1. INJURY TO APPLES FOLLOWING ANAEROBIC ZYMASIS.

(i) *Invasive alcohol poisoning.* It has been reported elsewhere (8) that whereas zymasis does not occur in healthy apples stored in CO_2-free atmospheres containing more than 5 per cent. oxygen, it commences when the external oxygen concentration falls below some value between 1 and 5 per cent., and is of a kind similar to that which takes place in the complete absence of oxygen. Such anaerobic zymasis continues so long as some of the cells remain alive, and is characterised by a relatively great accumulation of ethyl alcohol, and a small accumulation of acetaldehyde within the tissues. From Section 2, Table II, it will be seen that in apples which have been stored in 100 per cent. nitrogen, the ratio ethyl alcohol/acetaldehyde is usually $> 50/1$. Injuries to, and death of, the cells of an apple generally accompany these metabolic changes if anaerobic conditions are maintained for long enough. Progressive stages of these injurious effects are shown on Plate XX, figs. 1 *a* and 1 *b*. It should be noticed that injury is usually incident in the epidermal cells, and progresses inwards, until finally the whole apple may be killed. The photographs were taken some time after removal from the anaerobic conditions to air, so the injured and killed tissue had turned brown. Acetaldehyde ceases to accumulate in apples some time before injury is apparent and indeed before the rate of ethyl alcohol formation by anaerobic zymasis is retarded to any extent (8). Further, since the data which will be presented in Section 2 indicate that acetaldehyde in concentrations less than 0·01 per cent. is not toxic to apple cells, and since the amount of this substance has never been found to exceed 0·006 per

[1] Results are expressed throughout in terms of gms. acetaldehyde or ethyl alcohol found in 100 gms. fresh weight of the tissue of an apple or a sample of apples.

cent. after anaerobic zymasis, there is no evident connection between acetaldehyde and the injuries occurring under anaerobic conditions. The results given in Table I, however, suggest that there may be a relation between such injuries and the concentration of ethyl alcohol found by analysis in apples removed from anaerobic stores.

Table I.

On the relation between ethyl alcohol production and invasive alcohol poisoning in anaerobic storage.

Temperature of storage chamber	No. of days in anaerobic store before invasive alcohol poisoning noticed	Percentage of ethyl alcohol in apples showing invasive alcohol poisoning
22° C.	5	0·368
15° C.	9–13	0·396–0·504
1° C.	38	0·389

Although the data are limited in number, we are probably justified in concluding from this table that an accumulation of ethyl alcohol of the order 0·3 per cent. will, at all temperatures, precede the commencement of injury to apple tissue under anaerobic conditions, and so of the appearance of browning effects when such injured apples are subsequently exposed to air. Since we shall in the next section describe injuries incident at the surface of apples and progressing inwards which are brought about by quite different conditions, it is proposed to speak of the type of injury following a deficiency of oxygen alone as "invasive alcohol poisoning." It is not asserted that ethyl alcohol is the sole cause of these injuries[1], but it certainly appears to be the best index of the general state of poisoning which follows anaerobic zymasis.

(ii) *Diagnosis by chemical analysis of invasive alcohol poisoning.* It is probable that invasive alcohol poisoning can be diagnosed as such by chemical analysis of apples, because concentrations of ethyl alcohol of the order 0·3 per cent. have never been found in apples excepting after anaerobic storage. We shall see in the next section that it may be further distinguished from the invasive injuries resulting from an excess of carbon dioxide in the presence of oxygen, by the value of the ethyl alcohol/acetaldehyde ratio. Since neither ethyl alcohol nor acetaldehyde is oxidised by apple cells, and since these substances are not lost by evaporation with any considerable degree of rapidity (8), it is permissible to delay the analyses of apples suspected to be suffering from invasive alcohol poisoning for several days after removal from the conditions

[1] Attempts to produce differential browning in halved apples placed in the vapour of ethyl alcohol failed. The surfaces of the apples, if they were quite healthy at the start, browned uniformly.

which induced zymasis, provided that micro-organisms have not subsequently changed the products of metabolism. This is an important point, for it follows that apples arriving from overseas in a damaged condition may be held in store for a short time before being analysed, without necessarily invalidating the results of the analysis. Further, all the results so far obtained indicate that anaerobic zymasis occurs at the same rate and to the same extent in all parts of the flesh tissue of a single apple[1]. Hence, analysis of the remaining sound parts of an apple, suspected to be suffering from invasive alcohol poisoning, will be sufficient for diagnostic purposes, and will certainly, in a delayed analysis, give more trustworthy results than those which would be obtained from damaged parts in which micro-organisms will probably have been active.

SECTION 2. INJURIES TO APPLES FOLLOWING CO_2-ZYMASIS.

(i) *Internal aldehyde poisoning and invasive aldehyde poisoning.* A full account has been given elsewhere(8) of the discovery that carbon dioxide above certain concentrations, even in the presence of abundant oxygen, may cause the respiratory metabolism of glucose by apple cells to be changed to a zymasic type. Although the end products of this CO_2-zymasis are the same as those of anaerobic zymasis, it has been found that during CO_2-zymasis more acetaldehyde and correspondingly less ethyl alcohol accumulates in the cells. Indeed, during and after CO_2-zymasis the ratio ethyl alcohol/acetaldehyde is approximately 2/1—in striking contrast to the ratio 50/1 already recorded as characterising anaerobic zymasis. The continuous accumulation of acetaldehyde during CO_2-zymasis, which leads to the differences which have just been noted, is correlated with the presence of oxygen in the environment.

The toxicity of acetaldehyde is greater than that of ethyl alcohol, so it is not surprising that gas mixtures containing both carbon dioxide and oxygen may prove more harmful to apples than anaerobic conditions whether imposed by using pure carbon dioxide, pure nitrogen, or any mixture of these two gases. After CO_2-zymasis, injury which is accompanied by browning, since oxygen is present, is usually first incident in the interior (see Plate XX, fig. 2 *a* and Table II (A)), and not until later does invasive browning from the epidermal cells inwards occur (see Plate XX, fig. 2 *b* and Table II (B)); finally the whole apple may be killed (see Plate XX, fig. 2 *c* and Table II (D)).

[1] It seems from this, that the incidence of invasive alcohol poisoning in the epidermal cells of apples must be ascribed to the greater susceptibility of these cells to the adverse consequences of anaerobic conditions. Incidence of alcohol poisoning in the interior, to give "internal alcohol poisoning," is of course possible but has not yet been encountered.

The results presented in Table II were obtained from experiments in which apples were subjected to chemical analysis after storage at two different temperatures for different time periods in gas mixtures of carbon dioxide and oxygen, or under anaerobic conditions. These results serve for the comparison of the toxic effects resulting from CO_2-zymasis with those following anaerobic zymasis, whether induced by pure nitrogen or pure carbon dioxide.

Table II.

Comparison of toxicity of CO_2*-zymasis and anaerobic zymasis.*

Temperature 1° C.

Days in storage chamber	Gas mixture of 50 % carbon dioxide + 50 % oxygen in storage chamber		100 % nitrogen in storage chamber	
	Ethyl alcohol %	Acetaldehyde %	Ethyl alcohol %	Acetaldehyde %
3	—	0·01	—	—
7	0·05	0·02 (A)	—	—
15	0·075 (B)	0·03 (B)	0·17	0·0035
23	0·08 (D)	0·04 (C)	0·25	0·004
38	0·08 (D)	0·04 (D)	0·39 (C)	0·004 (C)

Temperature 15° C.

Days in storage chamber	Gas mixture of 70 % carbon dioxide + 30 % oxygen in storage chamber. Acetaldehyde %	100 % carbon dioxide in storage chamber. Acetaldehyde %
3	0·005	—
5	0·010 (A)	0·005
11	0·030 (B)	0·006
20	— (D)	0·006 (C)

(A) Visible browning in the interior. "Internal aldehyde poisoning" (see Plate XX, fig. 2 *a*).

(B) Visible browning incident in epidermis and progresses inwards. "Invasive aldehyde poisoning" (see Plate XX, fig. 2 *b*).

(C) Injury incident in epidermis and progresses inwards. Injured tissue browns on subsequent exposure to oxygen. "Invasive alcohol poisoning" (see Plate XX, fig. 1 *a*).

(D) Apples completely killed (see Plate XX, figs. 1 *b* and 2 *c*).

It will be seen that at both temperatures the apples used were more quickly injured and killed in gas mixtures of carbon dioxide and oxygen than when under anaerobic conditions. Further, at 1° C. after CO_2-zymasis apples may be completely killed when they contain only 0·08 per cent. ethyl alcohol, whereas apples containing 0·25 per cent. ethyl alcohol after anaerobic zymasis generally show little or no sign of

injury when placed subsequently in air. So it seems to be very significant that when injury is first seen after CO_2-zymasis at both temperatures (injury A), there is present in the tissues a greater concentration of acetaldehyde (0·01–0·02 per cent.) than has as yet been found to accumulate by anaerobic zymasis. Not all apples show this internal injury after CO_2-zymasis, and it is important to notice that it has never been encountered after anaerobic zymasis; two findings, which are in accord with the conclusions of Kidd and West, are that "brown-heart" only occurs in apples stored in chambers containing carbon dioxide and oxygen, and that the susceptibility of apples to this disease varies greatly (3). A consideration of the invasive injuries (injuries B and C), which occur after both these unusual respiratory processes have been in progress for some time, leaves little doubt that at both 1° C. and 15° C. their earlier appearance and more rapid spread in gas mixtures of carbon dioxide and oxygen may be correlated with the accumulation, under these conditions, of an inducing concentration of 0·03–0·04 per cent. acetaldehyde in the tissues, many days before the products of anaerobic zymasis have reached a toxic limit in apples placed under anaerobic conditions for the same time periods and at the same temperatures. Indeed so poisonous is acetaldehyde that apples are, as a rule, completely killed when 0·04 per cent. has accumulated in them.

It seems probable that both the internal and invasive brownings, which occur after CO_2-zymasis in apples stored in carbon dioxide and oxygen, are actually caused by the acetaldehyde which is produced. Support is given to this opinion by the results of experiments on apples which were first halved by transverse cuts, and then placed in covered desiccators at 16–18° C. over freely evaporating acetaldehyde: after 24 hours internal flesh browning was seen in some of the apples (see Plate XX, fig. 3 *a*), although during this period very little carbon dioxide had accumulated in the desiccators; and, later, invasive browning occurred (see Plate XX, figs. 3 *b* and 3 *c*). Clearly the localisation and succession of injuries in halved apples in pure acetaldehyde vapour is the same as when this substance is formed within the cells by CO_2-zymasis. Hence it is thought that we may rightly speak of the injuries occurring in apples during CO_2-zymasis as "internal aldehyde poisoning" (Table II (A)) and "invasive aldehyde poisoning" (Table II (B)) (cf. remarks in Section 1 on "invasive alcohol poisoning").

Evidently the differential browning produced by acetaldehyde vapour is due to the different susceptibilities of the cells of apples to this substance. Suggestions, supported by analyses of the internal

atmosphere of apples, have been made that the deep-seated and localised incidence of the injury called brown-heart may be due to the danger limit of carbon dioxide being reached earlier in certain localities within this bulky fruit (3). This explanation, however, will not hold for all cases of internal aldehyde poisoning after CO_2-zymasis, as halved apples placed in 50 per cent. carbon dioxide + 50 per cent. oxygen may show the same sequence of injuries as are seen in whole apples after CO_2-zymasis (see Plate XX, figs. 2 *a*, 2 *b* and 2 *c*). It seems to be better to think of the differential browning following CO_2-zymasis as due to the different susceptibilities of the cells of apples to the acetaldehyde which is produced.

(ii) *Diagnosis by chemical analysis of internal aldehyde poisoning and invasive aldehyde poisoning.* It may in future be possible to diagnose by chemical analysis internal aldehyde poisoning in apples brought to the laboratory for examination, and which on cutting show internal browning effects, since values of acetaldehyde of the order 0·01 per cent. or more will only be given if CO_2-zymasis has occurred. Certainly, as the figures assembled in Table III show, it should always be possible to distinguish invasive aldehyde poisoning from invasive alcohol poisoning.

Table III.

Distinction between invasive aldehyde poisoning and invasive alcohol poisoning by chemical analysis.

	After invasive aldehyde poisoning appears	After invasive alcohol poisoning appears
Concentration of ethyl alcohol in apples	Usually $<0{\cdot}1$ %	Usually $>0{\cdot}3$ %
Concentration of acetaldehyde in apples	Of the order 0·03 %	Usually $<0{\cdot}006$ %
Ethyl alcohol/acetaldehyde	Of the order 2/1	$>50/1$

The numbers relating to invasive alcohol poisoning seem to be independent of the gas in which anaerobic zymasis occurs: for example, they hold good for apples kept in pure nitrogen or pure carbon dioxide. The numbers relating to invasive aldehyde poisoning have only been obtained in gas mixtures of carbon dioxide and oxygen, and they appear to be quite independent of the concentration of carbon dioxide, provided this is sufficient, in the first place, to induce zymasis (8).

(iii) *Aldehyde poisoning in relation to the physiological state of apples, and to the concentration of carbon dioxide and oxygen. Gas storage.* In these experiments CO_2-zymasis occurred practically at once in all apples placed in gas mixtures containing more than 50 per cent. carbon dioxide,

provided that at least 5 per cent. oxygen was present[1], and under these conditions the apples were poisoned very quickly by the acetaldehyde which was produced. Considerable variation of resistance towards concentrations of carbon dioxide which were less than 30 per cent. was, however, shown[2]; some apples underwent CO_2-zymasis and showed the injurious after-effects of this more rapidly, and others more slowly (8). Further evidence of the variation of the resistance of apples towards carbon dioxide has been given by Kidd and West in their report on brown-heart (3); and also by workers in the U.S.A. on the development of "disagreeable alcoholic flavours" and of tissue browning in apples placed in gas mixtures containing carbon dioxide and oxygen (6). Possibly the cell metabolism of apples is disordered by progressively lower concentrations of carbon dioxide as the apples become more senescent. If this is so, then the temperature of storage and the concentration of oxygen in the store will both be of great importance in relation to these phenomena, for there can be little doubt that the rate of the march of senescence can be accelerated, within limits, by raising the temperature of the store, or by increasing the oxygen concentration in it. In this connection the following results of analyses are significant:

Exp. (*a*). Apples gave no products of zymasis after "gas storage" for 6 months at 8° C. in a gas mixture containing 10 per cent. carbon dioxide, 10 per cent. oxygen and 80 per cent. nitrogen (see Introduction).

Exp. (*b*). A gas stream containing 12 per cent. carbon dioxide and 88 per cent. oxygen was passed over apples, which had previously been stored in air at 1° C. for 6 months, placed in desiccators at 15° C. Zymasis occurred after 21 days, and injurious effects were seen later. It is suggested that during the 21 days the senescence processes in the apples were so hastened by the high concentration of oxygen acting in conjunction with the increased temperature, that the resistance of the apples to carbon dioxide was rapidly lowered, and finally zymasis occurred.

Although Exp. (*b*) is not a strict control for Exp. (*a*), nevertheless these experiments when considered together indicate that subnormal concentrations of oxygen are effective in maintaining the resistance of

[1] With concentrations of oxygen <5 per cent. the average metabolism of the whole apple is expressed by numbers intermediate between those characterising anaerobic zymasis and CO_2-zymasis (8).

[2] No experiments have as yet been carried out with concentrations of carbon dioxide between 30 per cent. and 50 per cent.

apples to moderate strengths of carbon dioxide; hence, the efficiency of gas storage as practised by Kidd and West (see Introduction).

We conclude that there are some conjunctions of carbon dioxide and oxygen in which apples may be kept without zymasis or injury occurring, but that there are others in which all apples will show zymasis followed by aldehyde poisoning: in conjunctions intermediate between these, apples will respond according to their physiological states.

(iv) *Brown-heart as a form of injury which is possibly different from internal aldehyde poisoning.* In the course of their preliminary gas storage trials with apples, Kidd and West noticed that an injury, which they have called "brown-heart," sometimes occurred when the concentration of carbon dioxide in the storage atmosphere exceeded 13 per cent. (3). After the discovery of the phenomenon of CO_2-zymasis, it was naturally attractive to think that brown-heart injury is preceded by and caused by the formation, possibly locally, of acetaldehyde in apples under such conditions. Analyses of apples which had suffered during gas storage trials from brown-heart gave no evidence, however, that zymasis had occurred during the storage period. Since the injured tissue was shrivelled into a dry mass when the analyses were made, it is conceivable that zymasis had occurred, possibly locally, a considerable time previously, and that the products of zymasis had in the interval disappeared from the apples by diffusion or through some other cause. Further, Australian apples, suffering from what seemed to be typical brown-heart, have been analysed when the brown injured tissue was still soft and very moist. Small amounts of ethyl alcohol (0·09 per cent.) and acetaldehyde (0·004 per cent.) were recovered. Since, as will be shown in a later paper, some form of zymasis follows many different forms of injury to apple tissue, it is difficult at present to say whether the alcohol and aldehyde found in these injured Australian apples was produced before or after the commencement of brown-heart. Evidently it is not, as yet, proven that brown-heart is a form of internal aldehyde poisoning. Further work is required, and has been planned, to attempt to determine whether carbon dioxide in conjunction with oxygen injures apple tissue either directly or indirectly other than by the induction of CO_2-zymasis.

Summary and conclusions.

(1) In an earlier paper it was shown that ethyl alcohol and acetaldehyde, which are only present in traces in healthy Newton Wonder apples stored in air, are both formed during the respiratory processes which occur under anaerobic storage (*i.e.* by anaerobic zymasis), or

under the influence of certain mixtures of carbon dioxide and oxygen (*i.e.* by CO_2-zymasis). In that paper it was pointed out that both anaerobic zymasis and CO_2-zymasis were lethal processes, and that CO_2-zymasis was more lethal than anaerobic zymasis since in the former acetaldehyde, which is more toxic than ethyl alcohol, accumulates in greater concentration than in the latter(9).

(2) In this paper the poisoning of apples of this variety by the products of zymasis has been considered in greater detail.

(*a*) Under anaerobic conditions these apples sooner or later will suffer from what has been called "invasive alcohol poisoning," associated with, although not necessarily caused by, an accumulation by anaerobic zymasis of more than 0·3 per cent. ethyl alcohol in the tissues. Never more than 0·006 per cent. acetaldehyde has been found in apples suffering from this injury, and the ethyl alcohol/acetaldehyde ratio is usually $> 50/1$. The incidence of this injury at the surface and its regular progress inwards are ascribed to the different susceptibility of the cells of apples to the adverse consequences of anaerobic zymasis.

(*b*) In gas mixtures containing carbon dioxide and oxygen, these apples either at once (if the concentration of carbon dioxide > 50 per cent.), or in variable fashion according to their senescent states (if the concentration of carbon dioxide is < 30 per cent. and > 20 per cent.), undergo CO_2-zymasis, which is followed usually by "internal aldehyde poisoning" (when the concentration of acetaldehyde formed is $> 0{\cdot}01$ per cent.), and by "invasive aldehyde poisoning" (when the concentration of acetaldehyde formed is $> 0{\cdot}03$ per cent.). Never more than 0·08 per cent. of ethyl alcohol has been found in apples suffering from either form of aldehyde poisoning, and the ethyl alcohol/acetaldehyde ratio is of the order 2/1. Evidence is given that these injuries are actually caused by acetaldehyde, and that their incidence in different parts of apples is due to the different susceptibility of the cells of apples to this substance.

(3) In gas mixtures containing carbon dioxide and oxygen, if the concentration of carbon dioxide is < 13 per cent. (circa) and the concentration of oxygen is sub-normal, analyses of several varieties of apples indicate that zymasis does not occur; indeed, in such gas mixtures the storage lives of apples seem to be prolonged.

(4) With gas mixtures, containing carbon dioxide and oxygen, in which the concentration of carbon dioxide is > 13 per cent. and < 20 per cent., a type of internal browning called brown-heart sometimes occurs. Since it is still doubtful whether brown-heart is or is not a form of internal

aldehyde poisoning, experiments have been planned to determine more critically the relationship between brown-heart and zymasis.

(5) It may be possible in the future to diagnose invasive alcohol poisoning, and internal and invasive aldehyde poisoning, by determining the ethyl alcohol and acetaldehyde content of apples suspected by their appearance to be suffering from one or more of these injuries. Such analyses may be carried out many days after the occurrence of these injuries, since neither ethyl alcohol nor acetaldehyde is oxidised by apple cells, nor do they escape rapidly from apples. If these injuries were found in apples from stores in this country, or after transportation from overseas, it would follow from what has been said that the stores or ships' holds had not been properly ventilated. Indeed even if the apples are healthy any evidence of zymasis would point to the same conclusion. So analyses of this kind may prove useful in the future, if cases arise in which apples are suspected to have been damaged through neglect of rules as to ventilation.

Acknowledgments.

I wish to record my gratitude to Dr Franklin Kidd and Dr Cyril West of the Low Temperature Research Station, Cambridge, for their valuable criticisms during the progress of the work, and during the preparation of this paper. Some of the quantitative results which have been presented were obtained in the biochemical laboratory, Cambridge, and I am grateful to Prof. Sir F. G. Hopkins for the facilities given. To Mr Percy Gibson of the Botanical Department, Armstrong College, I am indebted for his willing assistance in taking photographs, and in many other ways.

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Fig. 1 (*a*).

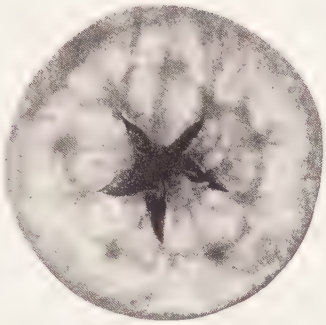

Fig. 1 (*b*).

Fig. 2 (*a*).

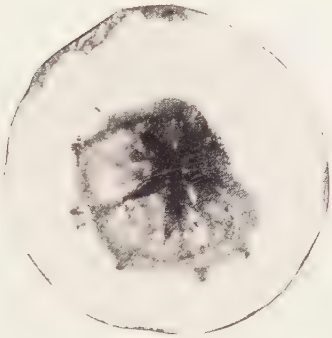

Fig. 2 (*b*).

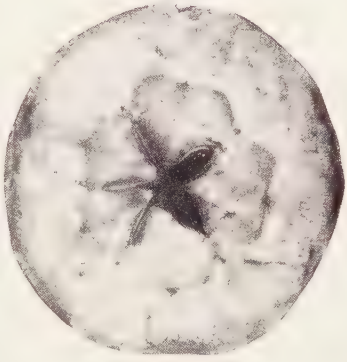

Fig. 2 (*c*).

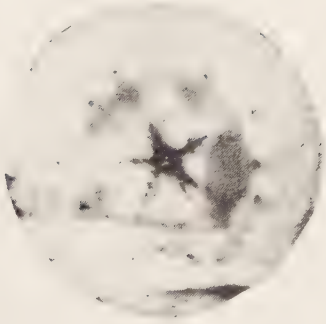

Fig. 3 (*a*).

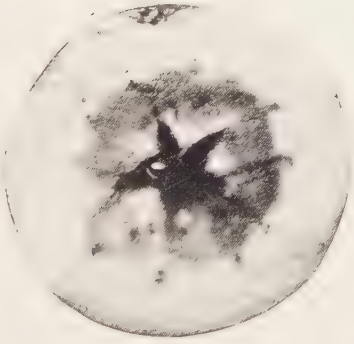

Fig. 3 (*b*).

Fig. 3 (*c*).

THOMAS.—Apples in Relation to the Injuries occurring in Storage (pp. 444–457).

EXPLANATION OF PLATE XX.

Figs. 1 *a* and 1 *b*. APPLES AFTER REMOVAL FROM ANAEROBIC CONDITIONS TO AIR, SHOWING EFFECTS OF INVASIVE ALCOHOL POISONING (see Section 1 (i)).

Fig. 1 *a*. Injury is incident in epidermal cells and progresses inwards.

Fig. 1 *b*. Finally the apple may be completely killed.

Figs. 2 *a*, 2 *b* and 2 *c*. BROWNING EFFECTS OF ALDEHYDE POISONING FOLLOWING CO_2-ZYMASIS, EITHER IN WHOLE APPLES OR IN APPLES HALVED BY TRANSVERSE CUTS (see Section 2 (i)).

Fig. 2 *a*. Internal aldehyde poisoning, incident in the heart tissue, sometimes brought about by high concentrations of carbon dioxide in the presence of oxygen.

Fig. 2 *b*. Invasive aldehyde poisoning which is always produced in high concentrations of carbon dioxide in the presence of oxygen, and which appears after internal aldehyde poisoning, when this occurs. Here internal aldehyde poisoning is very severe.

Fig. 2 *c*. The apple may be completely killed by internal and invasive aldehyde poisoning.

Figs. 3 *a*, 3 *b* and 3 *c*. BROWNING EFFECTS PRODUCED BY THE VAPOUR OF ACETALDEHYDE ON APPLES HALVED BY TRANSVERSE CUTS (see Section 2 (i)).

Fig. 3 *a*. After short exposure to the vapour, showing the internal incidence of browning in the "heart" tissue (cf. Fig. 2 *a*).

Fig. 3 *b*. After longer exposure showing more intense "heart" browning, and commencement of invasive browning (cf. Fig. 2 *b*).

Fig. 3 *c*. The apple is eventually completely killed.

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THE INTERNAL CONDITION OF THE HOST PLANT IN RELATION TO INSECT ATTACK, WITH SPECIAL REFERENCE TO THE INFLUENCE OF PYRIDINE

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PART I. THE NATURE OF THE PROBLEMS INVOLVED[1].

By J. DAVIDSON.

The study of the internal condition of the food-plant in relation to insect attacks has received very little attention on the experimental side, and a wide field is open for research on these lines. After a lengthy investigation of the factors affecting the infestation of the tea plant by *Helopeltis theivora*, Andrews (1) concluded that the vitality of this insect is directly controlled by the suitability or otherwise of the food supply. Withycombe (11) in Trinidad has shown that sugar cane growing on heavy clay soils, having badly aerated roots and a marked susceptibility to water shortage, more readily succumbs to the attack of the frog-hopper *Tomaspis saccharina*. Davidson (3) showed experimentally that there was a marked variation in the intensity of reproduction of *Aphis rumicis* on *Vicia faba* depending upon the nutrients supplied to the plants. Lees (5) refers to several instances which indicate the varying susceptibility of plants to insect attack due to the influence of soil conditions and growth of the plant.

The association of particular species of insects with particular food plants has doubtless resulted in an adaptation on the part of the insect, especially with regard to the physiology of digestion, in a manner best suited to its requirements. Many insects fail to live, or at any rate develop abnormally and only multiply at a markedly reduced rate, on

[1] The observations and experimental work on this subject were made at the Rothamsted Experimental Station while the writer was a member of the staff of the Entomological department.

other than their normal food plants as the writer (2) has shown to be the case with the polyphagous aphid *A. rumicis*. In some cases the specificity of the food plant is so definite that certain insects inhabiting one variety of a plant will fail to live on a closely related variety. One of the best known instances of this is the resistance of certain varieties of vines to Phylloxera. Trägardh, as referred to by Müller (6), records that apple trees may differ from 2·5 to 80 per cent. in the degree to which they are attacked by *Argyresthia conjugella*.

The resistance or immunity of a plant to insect attack may be due to particular features of its morphology or anatomy. In most instances, however, it appears to be due to features closely associated with the physiology of the plant, probably the presence or absence of particular substances in the tissues of the plant which render it unsuited to the insect. For instance, the larvae of *Pieris brassicae* and *P. rapae* appear to feed on those plants whose leaves contain a particular glucoside, one of the mustard oils, and it has been shown by Verschaffelt that when this substance was smeared on leaves which the caterpillars normally avoid, they were attracted to feed on them. The chemotropic influence and other factors which enable adult insects to select their appropriate food plants is little understood, and would be a profitable line of enquiry.

It is interesting to note that certain alkaloids, which when extracted from plants are toxic to insects, are not so in their natural state in the living plant. For instance, the tobacco plant is visited by cut worms, caterpillars and plant-sucking insects, yet nicotine which is extracted from it is a potent insecticide.

It may well be that the presence of certain chemical substances in the juices of plants render them unsuited to the requirements of certain insects, but no clear experimental proof of this with reference to insect-attack, so far as I know, has been brought forward. By inoculating the juices from one plant containing particular chemical compounds into another plant known to be lacking in those substances, one might expect the substances would be taken up into the juices of the latter plant, at any rate in so far as its physiological reaction would allow of this taking place. With this point of view, Müller (6) injected extract of tomato rhubarb and tobacco sap into the stem of an apple tree infested with *Eriosoma lanigerum* in May and June, but there was no evidence that the aphid was affected by this treatment.

Grafting would appear to be a more reliable method of obtaining a transference of particular substances from one plant to another. Roach (8), who cites several authors, states, "It is known that grafting together

portions of two different plants is sometimes followed by changes in their chemical contents owing to permeation of each by substances formed in the other." He refers to the work of Brown (1926), who showed that the mineral composition of the fruit of the apple varies according to the stock on which the variety is grafted, and Barker and Grove (1914), who found that the acidity and probably the tannin content are similarly affected.

The so-called "*incompatibility*" between scion and stock, when for instance certain varieties of pears are grafted on certain quince stocks, which is being investigated at East Malling Research Station is of interest in this respect. The results of research on the physiological and biochemical aspect of grafting should be of great interest to entomologists in relation to the association of certain insects with particular varieties of fruit trees.

In a recent paper Dontaho Kostoff(4) states: "In grafting certain species and genera of the Solanaceae, mutual induction of antibodies in scion and in stock was found." "Physiological interaction between scion and stocks accompanied by different morphological phenomena in the scions and stocks were observed, which are explicable by postulating antibody production."

From the entomological standpoint, attempts to confer on plants temporary immunity from insect attack have been based on the view that the plant may be induced to take up, and absorb into its tissues, traces of certain chemical substances which, although innocuous to the plant concerned, would by their presence render it unsuited or even toxic to the insects infesting it.

It is clear that the results of any attempt to influence the growing plant as indicated above must be closely bound up with the physiological processes of the plant, and it is not surprising to find that conflicting results have been obtained by different investigators. In a recent monograph entitled *Die innere Therapie der Pflanzen*, A. Müller(6) has reviewed the subject very thoroughly and shown how complicated are the many factors concerned. This monograph is a noteworthy contribution to a wide subject which has been comparatively little explored.

The methods which have been employed by various workers in introducing chemical substances into plants may be briefly grouped under two headings:

(*a*) By injection of substances in solution into the stem or roots of plants (usually trees) or by introducing solid substances (*e.g.* potassium cyanide) into holes in the stem.

(*b*) By introducing the substances either as solids or as solutions into the medium in which the plant is growing, the substances being taken up in the usual way through the roots of the plant.

The former method has been used by several workers with the object of ridding trees of borers, aphids, and scale insects. In the case of solutions, holes are bored into the stem in which a tube is inserted, the solutions being led into the tree from a suitable reservoir. The staining of the wood of living trees on this principle has long been practised and various types of apparatus have been devised for introducing solutions into trees. Under the influence of the transpiration current, a fairly uniform distribution of the solutions throughout the tissues is obtained. The wood when seasoned is used for making furniture. Aniline dyes and those of the phenol group have been chiefly used at concentrations of 0·5 to 1 per cent., about 600 litres of solution being required per cubic metre of the tree. Dementiev (*Abstr. R.A. Entom. A.* III, 1915, 394) introduced a solution of barium chloride 1 : 350 into apple trees infested with woolly aphis in July 1903. He records that after nine days the aphids had been practically all killed. Müller(6) tried similar methods against *E. lanigerum* using solutions of chloral hydrate, nicotine muriate, and pyridine. The latter gave good results. Marked branches of apple trees treated with pyridine on 8. vi. 22 were found to be freed from the aphids on 7. vii. 22.

Employing similar methods, other chemical substances in solution have been tried by different workers, but in many cases the results have either been negative, or the resulting damage to the plants, due to the treatment, has ruled out the method as of no practical value. It is clear, however, that meteorological conditions, time of the year (season), age and type of tree, and concentrations of the substances used, are important factors affecting the results obtained.

The introduction of solid substances into the stem of the plant has the advantage of being a convenient and simple method. Sanford (*Science*, Oct. 9th, 1914, No. 1032, 519) treated Spanish broom infested with *Icerya purchasi* by boring a hole three inches deep, diameter three-eighths inch, into the stem and filling it with crystals of potassium cyanide. In a few days the scale was killed and the tree flourished. Shattuck (*Science*, Feb. 26th, 1915, No. 1052, 324) states that large groves of elms and black locust trees were successfully treated by this method against attacks of borer and girdling insects. Moore and Ruggles (*Science*, July 2nd, 1915, No. 1070, 33) however obtained negative results with this method against wood borers in oak trees. Müller (p. 49)

refers to the work of Mokrzeki, who successfully treated apple and pear trees infested with *Lepidosaphes ulmi* and *Diaspis fallax* with iron sulphate solution.

The method whereby the chemical substances concerned are added to the soil in which the plants are grown is a more normal and convenient one. There are some interesting examples in the literature showing the curative effect on plants of adding particular substances to the soil. The best known case is probably that of chlorosis due to deficiency in the soil of iron or manganese. Samuel and Piper(9) have shown conclusively that the "Grey Speck" disease of oats in certain areas of South Australia is due to a manganese deficiency in the soil, and by the incorporation of manganese sulphate in the soil the disease can be completely cured. Andrews(1), working with *Helopeltis theivora* infesting the tea plant in North-east India, found "That when a constant supply of soluble potash is applied to the roots of the (tea) bush, this substance is taken up and the bushes which are entirely shut up by the pest can be caused to throw it off entirely, and remain immune from attacks for the rest of the season." Brenchley and Warrington at Rothamsted and others have shown the stimulating effect on plant growth of minute doses of certain chemical substances, *e.g.* Boron, which in slightly stronger doses are toxic to the plant.

Müller(6), working with "Blumenbohnen" in water culture, tested the effect on the plants of a range of substances in various concentrations. He found for instance that with pyridine 1 : 500 and barium chloride 1 : 5000, after 5 and 17 days respectively, no ill-effects were noted on the plants[1].

In 1924 the writer made some preliminary experiments in which solutions of various substances were inoculated by means of a hypodermic syringe into the stems of growing plants of *Vicia faba* infested with aphids. The following substances were used, strychnine, atropurine, digitalin, arsenic, potassium cyanide, nicotine, and pyridine. In several cases the substances were evidently carried through the plant in an irregular manner to the growing tip, and the aphids were killed. In some cases the plant itself was also killed, pathological symptoms first showing in certain areas of the growing tip, and as in the case of arsenic gradually spreading through the whole plant. Experiments were continued the

[1] With $MgSO_4$ 1 : 500 and chloral hydrate 1 : 1000, it was observed that the plants made better growth and bigger root development. The writer(3) working with *Vicia faba* in water culture in 1923 also obtained marked increase in root development and growth of the plants in solutions containing increased $MgSO_4$ as compared with the controls growing in normal culture solution.

following year by placing cut stems of *Vicia faba* plants infested with *Aphis rumicis* into solutions of various substances. The examples given in the following table of some results obtained at Rothamsted in July 1926 by Mr H. T. Pagden indicate the type of results obtained.

Solutions used	Plant no.	Date and time observations made: 28. vii. 10 a.m.	29. vii. 10 a.m.	29. vii. 6 p.m.	30. vii. 10 a.m.	30. vii. 6 p.m.
Tap water controls	1 2 3	Bean plants placed in solutions 10 a.m. Plants 12–14 in. in height	Plants and aphids normal	Normal	Normal, aphids quiet	Normal, aphids quiet
KCN 0·25 %	4 5 6		Aphids slightly wandering over plants; plants appear normal	Aphids sluggish; plants wilting and bending over about middle of stem	Aphids sluggish; plants wilting	Many aphids dead; did not wander, but died with stylets in tissues of plant
$BaCl_2$ 1 : 350	7 8 9		Aphids wandering, some dead; plants wilting	—	Some aphids dead, many wandering actively; plants with leaves badly blotched	—
$MgSO_4$ *M*/10	10 11 12		Plants and aphids normal	Plants normal; aphids slightly wandering	Plants appear normal; aphids wandering	Plants appear normal; aphids actively wandering over plants and muslin cover

Note. 100 c.c. of solution used in each case. Mean temperature throughout the period was 24·5° C. The amount of solution taken up by the plants varied. In the case of the control series, 50 c.c. were taken up during the first 24 hours, whereas in the case of the other solutions only 15–20 c.c. were taken up.

From preliminary experiments with pyridine, it was found that, when beans grown in sand culture and infested with *Aphis rumicis* were watered with pyridine solution 1 : 200 and 1 : 150, the pyridine was evidently taken up by the plant to the growing tip, and the aphids wandered from the plant about 12 hours later, the great majority of them being killed. During the first day or two after commencement of the experiment, the plants did not exhibit any marked effect of the pyridine. On examination of the roots after a few days it was found, however, that the fine rootlets were discoloured and dead. When the pyridine solution was watered to beans growing in soil, the effect on the aphids was not so quickly shown. In order to find out the strength of pyridine solution required to free the plants from the aphids and at the same time not to injure the plants, Mr Henson has carried out a series of experiments which are described in the second section of this paper. The results obtained indicate a useful line of enquiry in relation to the effect of pyridine and other chemical substances on the aphids and the physiological reaction of the plant.

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PART II. ON SOIL TREATMENT WITH PYRIDINE AND ITS EFFECT ON THE INFESTATION OF *VICIA FABA* BY *APHIS RUMICIS* L.

By H. HENSON.

Dr Davidson's preliminary experiments on this subject are referred to in Part i of this paper. They indicated that it was possible to administer a substance such as pyridine to a plant and that it would take this up *via* the roots and so render itself toxic to insect pests such as aphids, which live by sucking the plant juices. During the summer of 1928 a series of experiments were undertaken in order to further investigate this subject and to determine whether the method showed promise of being of any economic importance. Pyridine was chosen because it was known to give positive results and because its odour rendered its presence in the plants easily detectable. A wide field for investigation is offered by the possibility of using other substances. The whole work was done under the direction of Dr A. D. Imms and Dr J. Davidson, to whom my best thanks are due.

Three experiments were projected as follows:

(*a*) Administration of pyridine to infected broad beans under conditions which would prove whether or not a lethal effect, due to root absorption, could be obtained. It was also used as a preliminary investigation of the degree of concentration required.

(*b*) Administration of pyridine under conditions similar to the above, to determine whether a range of concentrations was available in which the pyridine was potent to the animal but harmless to the plant.

(*c*) An experiment in which beans not infected with aphids were given pyridine and then its effect determined by taking the dry weights of the plants treated, and of controls untreated. This experiment was also taken advantage of to determine whether a small amount of pyridine administered several times had a different effect from a larger amount administered once.

Experiment 1 (*a*).

Two series of beans were grown, one in soil and the other in sand. The sand cultures consisted of 48 pots divided into five series, four of ten each and one of eight. The pots were 9 × 11 in. (inside measurements) and were each fitted with a cork and glass drain tube. Any water which drained through was always returned to its own particular pot. Three days after planting (May 11th) each pot was given 1 gm. potassium nitrate, 1·24 gm. sodium sulphate, 0·4 gm. calcium chloride, 0·5 gm. magnesium sulphate, 0·5 gm. potassium dihydrogen phosphate and a trace of iron chloride made up in tap water. Subsequently the plants were only watered as required. On May 30th each plant was infected with one apterous viviparous female aphid (*A. rumicis* L.), the plants covered with muslin bags, and the infestation allowed to proceed. The commencement of the experiment for each pot was taken as the day on which the first young were produced. Apart from a few of the controls each pot was given 18 days as a reproduction period taking the day on which the first young were produced as the starting point. On June 11th pyridine was administered to 30 of the pots. The five series into which the 48 pots were divided were as follows:

1. Ten control pots in which petri dishes containing pyridine (1 part pyridine in 200 parts water) were put on the surface of the sand as a check on the action of the vapour given off from the treated pots.

2. Eight control pots in which dishes were not present.

3. Ten pots to each of which 500 c.c. of a $\frac{1}{2}$ per cent. solution of pyridine in water were added, *i.e.* $2\frac{1}{2}$ c.c. pyridine per pot.

4. Ten pots to each of which 500 c.c. of a $\frac{1}{4}$ per cent. solution of pyridine in water were added, *i.e.* $1\frac{1}{4}$ c.c. per pot.

5. Ten pots to each of which 500 c.c. of a $\frac{1}{8}$ per cent. solution of pyridine were added, *i.e.* $\frac{5}{8}$ c.c. per pot.

The controls were given 500 c.c. of water at the same time as the pyridine was administered to the other pots.

A table of the results is given below. The reproduction figures are given in the columns. Owing to pressure of time and the fact that pots were urgently required for the next experiments, some of the controls were examined before the expiry of their allotted 18 days. These are indicated in the columns.

Exp. 1 took place over the period May 31st–June 23rd. Mean temperature during this period was 60° F.

Table I.

	Controls		Pyridine administered		
	With vapour dishes	Without vapour dishes	$\frac{5}{8}$ c.c. per pot	$1\frac{1}{4}$ c.c. per pot	$2\frac{1}{2}$ c.c. per pot
1	388 (15 days)	365	0 d	0 d	0 w
2	391 (17 days)	385	9	0	0 w
3	398	396 (13 days)	15	0	0 d
4	413 (13 days)	411 (13 days)	46	0	0 d
5	421 (16 days)	—	59	2	0 w
6	510	566	66	6	0 w
7	518	—	70	12	0 d
8	820	747	75	23	0 d
9	878	782	169	32	0
10	Discarded	1043	295	58	4
Mean	526	587	80	13	—

It will be seen at once that the pyridine has a very marked effect on the infestation. In those cases in which the infestation was not entirely checked it seemed likely that it was due to reinfection after wandering of the aphids. Very numerous dead aphids were to be observed on all the plants which were given pyridine. The figures further indicate that the plant takes up the pyridine in direct proportion to the concentration in which it is administered.

Notes on the effect on the plants. Too much significance should not be placed on the following notes as the pyridine was in contact with the plant roots for a matter of five days or so, which would not be the case under field conditions. A small "w" in the columns in Table I indicates the plant to have been badly wilted. A small "d" indicates less severe damage. The roots were badly affected in all cases, being caused to go black and lose their turgidity. The effect was worse in the higher concentrations as would be expected. The wilting was probably correlated with cessation of absorption by the roots. The affected plants also tended to go lighter in colour. Of course the controls were not affected

in this manner. In a few plants the pyridine was washed out and the plants allowed to go on developing. One of these bore fruit and was still healthy on July 24th.

Experiment 1 (*b*).

Soil pots were treated in a similar fashion. Twenty-four pots were taken and divided into three series: (1) 8 controls; (2) 8 to which $\frac{5}{8}$ c.c. pyridine per pot was administered in 500 c.c. water; (3) 8 to which $2\frac{1}{2}$ c.c. pyridine per pot were administered in 500 c.c. of water.

Beans planted May 9th. Plants infected May 30th. Allowed 18 days as time of reproduction.

Results tabulated below (Table II).

Table II.

Soil series.

		Pyridine administered	
	Controls	$\frac{5}{8}$ c.c. per pot	$2\frac{1}{2}$ c.c. per pot
	1214	1202	50
	1211	915	24
	906	866	12 d
	882	733	11
	390	618	10
	214	209	3
	1281	128	2
	Discarded	Discarded	0
Mean	871	667	14

Mean temperature 60° F.

It will be seen that only one plant was badly affected. In fact all the others were apparently normal. The roots also were not nearly so badly affected as in the sand pots.

Exp. 1 proves that the pyridine is absorbed by the plants and, provided a suitable concentration is used, is lethal to the aphids. The concentrations to be used in Exp. 2 lie between $2\frac{1}{2}$ c.c. and $\frac{5}{8}$ c.c. per pot in the soil series, and between $1\frac{1}{4}$ c.c. and a lower limit not determined by Exp. 1 in the sand pots.

Experiment 2 (*a*).

In Exp. 2 (*a*), thirty sand pots were taken as before. The beans were planted in seed boxes on May 31st, transplanted to the pots on June 19th and culture media given to each pot as in Exp. 1, *i.e.* 1 gm. potassium nitrate, 1·24 gm. sodium sulphate, 0·4 gm. calcium chloride, 0·5 gm.

magnesium sulphate, 0·5 gm. potassium dihydrogen phosphate made up in tap water. On June 25th each plant was infected with a young apterous form and left to become infested. The date on which each mother first produced young was noted and the experiment was continued for 16 days (each pot) after this date. Pyridine was administered to each pot (in 500 c.c. of water) on the twelfth day (after infection) and left in contact for four days. The pots were then washed through with tap water.

Six series of five plants each were taken:

(1) Controls to which no pyridine was given.
(2) Five pots to which 0·1 c.c. pyridine was given.
(3) Five pots to which 0·2 c.c. pyridine was given.
(4) Five pots to which 0·5 c.c. pyridine was given.
(5) Five pots to which 0·6 c.c. pyridine was given.
(6) Five pots to which 1·25 c.c. pyridine were given.

During the period of the experiment the temperature was abnormally high and a high reproduction rate prevailed among the aphids. Under these conditions any slight variation in the action of the pyridine produced a much more noticeable effect on the counts due to the very rapid recovery after the check on the infestation. This is suggested as the explanation of the rather aberrant figures of 617 and 580 in two of the 1·25 c.c. pots. Further, it is probable that under the hot dry conditions the plants absorbed and quickly eliminated the pyridine. No data have been obtained with regard to variation in rate of absorption by the plants or of the conditions of their responses to the presence of the poison.

A table with the infestation figures is given as before.

Reproduction period 16 days. Mean temperature July 2nd–21st 70° F.

Controls	·1 c.c. per pot	·2 c.c. per pot	·5 c.c. per pot	·6 c.c. per pot	1·25 c.c. per pot
2869	1584	681	667	181	617
2655	1842	1311	290	50	580
2297	1691	1463	415	223	56
2562	1657	Plant no good	554	242	Plant died
2908	2757	1739	206	56	3
Mean:					
2658	1906	1298	426	150	314

The actual concentration per pot cannot be ascertained owing to the amount of water in each pot being unknown. The lowest concentration used was 0·1 c.c. per pot and was actually something less than 1 in 5000.

It will be observed that the same effect was obtained as in Exp. 1. The effect on the aphids is proportional to the concentration of the pyridine. This is the case even down to the very lowest concentrations used. No clear cut range of concentrations has been obtained in which the pyridine was lethal to the aphids but harmless to the plants.

The effects on the plants.

In no case was a detrimental effect on the plants noticed. As the highest concentration used was 1·25 c.c. per pot this agrees almost exactly with what was found in Exp. 1, where only one plant was damaged seriously by this concentration. From casual observance there seems to be a check on growth but no actual damage. Ten plants were washed clear of pyridine and left. These were still alive and healthy on August 18th.

From these data it would seem that two courses of action could be taken to check infestation: (*a*) a low concentration administered several times, or (*b*) a high concentration (represented by the 1·25 c.c. per pot conc.) allowed to act once.

Whichever of these alternatives is chosen will depend on the effect on the plants. Further data on this point will be obtained from the dry weight experiment.

Experiment 2 (*b*).

This was another soil pot experiment which gives results in accordance with what was discovered in Exp. 1 (*b*).

Reproduction period 16 days. Mean temperature 70° F.

	Controls	·8 c.c. per pot	1·25 c.c. per pot
	1496	538	544
	1123	227	1237
	333	2107	649
	1414	528	72
Mean	1091·5	850	625

Experiment 3.

The object of this experiment was to determine the effect of the pyridine on the plants. Beans were planted in sand pots on June 20th. On June 21st each pot was given a culture solution containing 3 gm. potassium nitrate, 3·72 gm. sodium sulphate, 1·2 gm. calcium chloride, 1·5 gm. magnesium sulphate, 1·5 gm. potassium dihydrogen phosphate (made up in tap water). Each pot had ample supplies of water.

Twenty-four pots were used and divided into three series as follows:

(1) Eight controls to which pyridine was not added.

(2) Eight pots to which 1·2 c.c. pyridine in 500 c.c. water were added on the fortieth day after planting (July 30th).

(3) Eight pots to which 0·2 c.c. pyridine in 200 c.c. water was added on six occasions, July 24th, 26th, 28th, 30th, August 1st and 3rd.

Each pot had a drain tube as in previous experiments and ample supplies of water were always present; the drain water was returned to the pot at intervals.

The results are given in the table as dry weights of the plants concerned.

Dry weights. Period of experiment June 20th–August 14th. Weight in gm.

	Controls	1·2 c.c. per pot given once	·2 c.c. per pot given six times
	1·9	1·45	1·55
	3·95	2·15	1·7
	4·2	2·85	2
	4·45	3	2·85
	5·3	3·5	3·9
	6·7	3·6	4·5
	8·3	4·7	4·95
	9·3	5·55	5·05
Total	44·1	26·8	26·5
Mean	5·5	3·35	3·31

It will be seen at once that a very marked effect on the plants is evident from their dry weights. This single experiment is not by any means conclusive, since the existence and extent of the subsequent recovery remain unknown and many more experiments are really required to settle these points. In this particular case no difference is to be observed between the series in which a large dose of pyridine was administered once and the series in which an equivalent amount was administered in successive small doses.

Conclusion.

It is known that it is possible to administer to the roots of broad beans certain substances which are absorbed and transferred to the leaves and stems. In experiments in which pyridine was used it is shown that this substance, in suitable concentrations, exercises a marked detrimental effect upon the aphids. There are many features which render the exact conditions rather difficult to define. The great majority of these features are concerned with the conditions governing absorption

by the plant and with the effect of the pyridine on the plant after absorption. It was noticeable in the sand experiments that the effect on the aphids was to a large extent proportional to the amount of pyridine administered to the plant. The experiment in which dry weights of treated and control plants were compared shows quite plainly that a very evident effect is caused by the pyridine. In experiments on plants growing in soil the pyridine appeared to have a much less detrimental effect on the plants. It still had a very obvious effect on the aphids when present in sufficiently high concentration. This concentration had to be much higher than was the case with plants grown in sand.

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CARBON DIOXIDE PRODUCTION IN SANDS AND SOILS IN THE PRESENCE AND ABSENCE OF AMOEBAE

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(With 2 Text-figures.)

If the functions of the micro-organisms in soil are to be fully understood a knowledge of their relation to the different chemical constituents of the soil is essential, so that the parts they play in breaking down or building up compounds can be evaluated. The present work was undertaken in an attempt to throw some light on the relation of bacteria and amoebae to carbon dioxide production and by this means to the organic matter in the soil. Such investigation is particularly interesting since it has been shown that amoebae are an important part of the active soil population and that their numbers are definitely related to those of the bacteria (4). Since the problem is one of great complexity only the simplest cases have hitherto been considered and the attempt has been confined to studying the action of one pure strain of bacteria, in the presence or absence of a pure line of amoebae, in sand cultures containing four different carbon sources, and in sterilised soils with four different manurial histories.

Previous work on carbon dioxide production from soil has largely been directed to attempting to correlate for field soils the carbon dioxide produced with their fertility, no effort being made to simplify the population; the literature of this subject is reviewed by Waksman and Starkey (15) and Waksman (14) and need not be considered here. All the work done has gone to show that carbon dioxide formation is conditioned by numerous factors, among which heat, moisture, source of carbon and type of organisms may be mentioned as the more important, and many investigators have concluded that it is safe to regard carbon dioxide production as a useful index of soil fertility, possibly of greater use in this connection than the actual numbers of bacteria present in the soil. Several observers (Stocklasa (12), Russell and Appleyard (10), Neller (7),

König and Hasenbäumer (6), van Suchtelen (13)) found a definite correlation between carbon dioxide production and bacterial numbers. The amount of carbon dioxide produced by an unsterilised field soil is very variable; we have found that a well manured soil such as the dunged plot on Barnfield can give from 18 to 157 mgm. per kilo for the first 24 hours of an experiment, and an unmanured, 12 to 32. Waksman and Starkey recorded over 100 mgm. from one kilogramme of soil from a well manured, limed plot, and about 20 mgm. from an untreated soil.

A certain amount of work has also been done with pure cultures of bacteria inoculated into field soils; Westhues (16) found that sterile garden soil inoculated with three different species of bacteria gave amounts of carbon dioxide varying from 136 to 274 mgm. per 500 gm. in seven weeks.

Very little work has been done on the respiration of protozoa, one can therefore lay down no *a priori* expectation as to the amount of carbon dioxide that they will be responsible for producing in the soil. The little that is known is mostly derived from work on Paramoecium. Barratt (1) calculated that Paramoecium gave off from 1·3 to 5·3 per cent. of its body weight per day, and Pütter (9) calculated that this would represent 0·000035 mgm. per animal per day, but it must be remembered that Barratt's animals were in distilled water so that metabolism was at a low ebb and the figures quoted are therefore minimum and not average.

Methods.

In the experiments under consideration both sands and soils were used as medium; the sand was silver sand which passed a 1 mm. sieve, it was washed with hydrochloric acid, and then with water until it was acid-free, and was then ignited. To the sand four different food sources were added in different experiments, after sterilisation, when the micro-organisms were inoculated. The four food solutions used were soil extract, mineral salt solution + 0·5 per cent. peptone, mineral salt solution + ammonium sulphate + 0·2 per cent. glucose (ratio C/N = 10), and mineral salt solution + sodium nitrate + 0·2 per cent. glucose (ratio C/N = 10).

The soils were taken from plots on Barnfield which have been under continuous cultivation since 1876, receiving respectively fourteen tons of farmyard manure, 550 lb. nitrate of soda and 400 lb. ammonium salts per acre per annum; the fourth plot received no manure. The soils all received the same treatment in the laboratory; they were air-dried, sieved through a 3 mm. sieve and the required amount was then autoclaved

in a two-litre flask for ten minutes at 15 lb. pressure. The sand was similarly autoclaved. Experience has shown that soil which is to be used as a medium for the growth of protozoa must not be subjected to a too drastic sterilisation, since toxic products are formed which inhibit growth (3). Pure cultures of "YB" bacteria (3) were employed, obtained from a single cell[1], either with or without the addition of a pure mixed culture of *Hartmanella hyalina* with "YB," and every experiment consisted of parallel cultures of bacteria alone and bacteria + amoebae, so that the results are strictly comparable. The media were inoculated under aseptic conditions by spraying the cultures of bacteria or bacteria and amoebae on to the sand or soil from an Atlas spray, sufficient food solution in the case of sands or mineral salt solution in the case of soils being added to bring the medium to half the water-holding capacity. Bacteria and amoebae were counted daily by the dilution method in use in this laboratory (4).

The carbon dioxide evolved was estimated by the Pettenkoffer method using a baryta solution of about 0·1 per cent. in the tubes and titrating it against known hydrochloric acid of not more than $N/5$ strength. In every case the cultures were aerated by drawing carbon dioxide free air over them continuously by an aspirator. It has been shown by Potter and Snyder (8) that within wide limits the rate at which the air is drawn over the soil is immaterial; in these experiments the rate was between four and five litres in 24 hours. The carbon dioxide was estimated at least once in every 24 hours, that is, when the bacteria and amoebae were counted, but frequently the amount produced was so great as to necessitate more frequent titrations.

Results.

The growth of bacteria in an inoculated sand or soil follows closely the growth in an ordinary liquid culture; the numbers rise to a maximum within the first five days and then fall steadily with minor fluctuations; the presence of amoebae induces a greater tendency to fluctuate and lowers the bacterial numbers. Contrary to expectation, in the majority of cases the carbon dioxide production reaches its maximum a day before the bacterial numbers have reached theirs; then it drops sharply at first and more and more slowly until a steady level is maintained, at least for the duration of the experiments under discussion, which is a period of anything up to 15 days. Typical curves showing the carbon

[1] This pure strain of bacteria was prepared for us by Mr R. H. Stoughton using the "Dickinson Micro-Isolator."

dioxide production are given in Figs. 1 and 2. The amount of carbon dioxide produced in any given period is conditioned by a variety of factors as has been previously stated, but a rough idea of the amount

Fig. 1. Carbon dioxide production from bacteria and bacteria + amoebae in sand cultures with peptone.

of carbon dioxide which any given medium is capable of producing may be obtained from Table I, in which the amount of carbon dioxide given off during the first five days of an experiment is given.

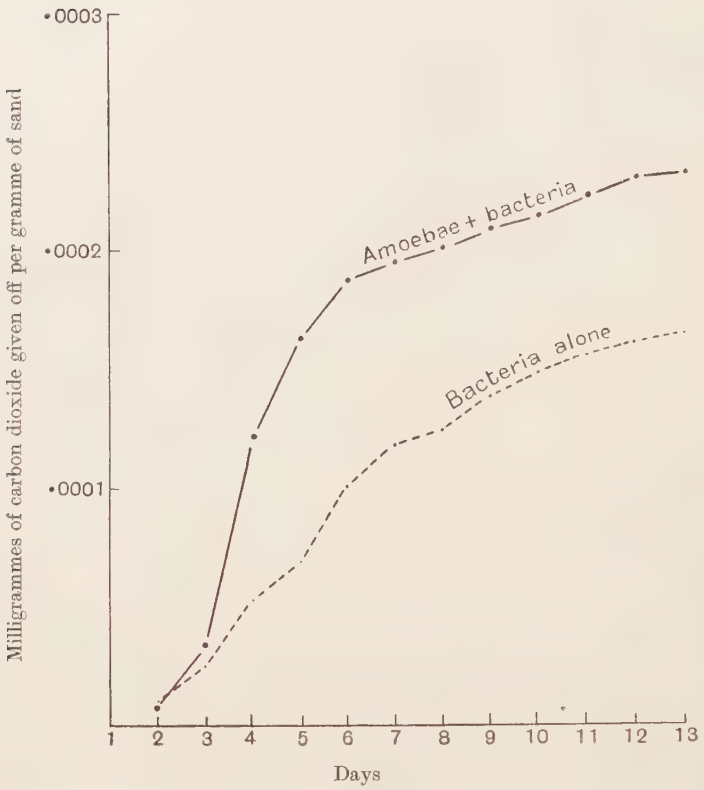
·0003
·0002
·0001
Milligrammes of carbon dioxide given off per gramme of sand
Amoebae + bacteria
Bacteria alone
1 2 3 4 5 6 7 8 9 10 11 12 13
Days

Fig. 2. Carbon dioxide production from bacteria and bacteria + amoebae in sand cultures with ammonium sulphate and glucose.

Table I.

Average amount in gm. of carbon dioxide produced from 400 *gm. of media in five days.*

Soils	Farmyard manured	Unmanured	$(NH_4)_2SO_4$	$NaNO_3$
	·1984	·0324	·0592	·0556
Sands	Peptone	Soil extract	$(NH_4)_2SO_4$	$NaNO_3$
	·1126	·0164	·0222	·0212

It is obviously of considerable interest to know what relation the numbers of bacteria bear to the formation of carbon dioxide; usually it has been assumed that there will be a definite relation between high numbers of bacteria and high carbon dioxide production and *vice versa*. The simplest method of testing this point since only two variables are involved is to arrange the data in contingency tables: in these an increase

in either variant is shown by a + sign and a decrease by a − sign; if both increase or decrease together it will be shown by a + + or a − −, if they vary inversely by a + − or − +. Where the two vary wholly independently there will be equality between the numbers of like and unlike signs, provided that there are a sufficient number of cases; a preponderance of like or unlike signs will show that the two variables are related to one another. To test the significance of any departure from equality a χ^2 may be worked out (5), and when a χ^2 has a greater value than 4 it may be assumed that there is a definite inter-relation between the two variables which is not due to chance. From Table II it appears

Table II.

Contingency tables for carbon dioxide production and bacterial numbers in sand and soil cultures (CO_2 *given first*).

Sands		+ +	+ −	− +	− −	χ^2
$(NH_4)_2SO_4$ or $NaNO_3$ +glucose	Bacteria alone	11	5	3	12	6·2
	With amoebae	10	4	6	13	5·1
Peptone	Bacteria alone	12	6	6	15	5·6
	With amoebae	10	8	10	12	0·4
Soil extract	Bacteria alone	15	7	4	11	6·1
	With amoebae	12	15	6	9	0·08
Soils		+ +	+ −	− +	− −	χ^2
$(NH_4)_2SO_4$ or $NaNO_3$	Bacteria alone	9	20	9	17	—
	With amoebae	11	12	8	13	—
Farmyard manured	Bacteria alone	12	6	4	23	12·9
	With amoebae	7	6	3	13	3·9
Unmanured	Bacteria alone	3	8	5	7	—
	With amoebae	1	3	2	3	—

that where bacteria alone are present in sand cultures there is a very definite correlation between their numbers and the amount of carbon dioxide given off. The same is true of the soil receiving farmyard manure, but in the case of the unmanured soil and the soils receiving minerals there is no such correlation. There is no obvious reason for this anomalous result.

It is a matter of considerable interest to discover whether the bacterial capacity for producing carbon dioxide varies at all in the course of growth, and on different media, or whether it remains a constant. It appears from a study of different experiments that the carbon dioxide is very often produced in large quantities at the beginning of an experiment, even where in some cases the numbers of bacteria are still low. A contingency table was therefore made in which the two variables considered were the numbers of bacteria per gramme of medium,

averaged for the beginning and end of each 24-hour period, and the amount of carbon dioxide produced per bacterium during that 24 hours, this giving a measure of the efficiency of the bacteria.

Table III.

Contingency table for numbers of bacteria and efficiency in producing carbon dioxide (efficiency given first).

Sands	+ +	+ −	− +	− −	χ^2
Soil extract	10	13	14	5	4·8
Minerals + glucose	4	10	8	7	1·9
Peptone	5	10	11	4	4·8
All media	19	33	33	16	9·5
Soils	**+ +**	**+ −**	**− +**	**− −**	**χ^2**
Farmyard manured	6	9	14	8	2·0
Unmanured	3	5	12	3	4·1
Minerals	6	12	27	11	7·1
All media	15	26	53	22	12·6

From Table III it is clear that in the majority of cases, both in sands and in soils, the bacteria are more efficient as producers of carbon dioxide when their numbers are not rising and less efficient as the numbers increase. That these results are not due to the fact that when low numbers of bacteria are present the culture is usually young and active and when high numbers are present it is staling, is shown in Table IV by considering the figures obtained when only the last five days of the culture's growth are considered.

Table IV.

Medium	+ +	+ −	− +	− −	χ^2
Peptone	1	10	2	2	3·1
Soil extract	3	8	7	1	6·7
Minerals + glucose	0	7	3	0	—
All media	4	25	12	3	25·2

The obvious interpretation to place upon these results is that when the bacteria are reproducing, their energy is primarily devoted to building up fresh tissue, and that when reproduction is at a standstill, the energy is diverted and carbon dioxide is released in much larger quantities. In the case of young cultures, there is the anomaly that high efficiency for carbon dioxide production is correlated with active reproduction; apparently the bacteria of young cultures are able both to reproduce and to liberate carbon dioxide at the same time, whereas in the older cultures one or the other of the two processes predominates. These

contingency tables simply demonstrate that the efficiency varies according as to whether the numbers of bacteria in the cultures are increasing or decreasing, but they afford no information as to whether the efficiency is affected by the actual density of the population or as to whether it varies from medium to medium. If the efficiencies are grouped as in Table V, according to the number of bacteria present per gramme of

Table V.

Bacterial efficiencies in gm. per 1000 *million bacteria.*

	Numbers of bacteria in millions per gramme									
	No. of cases	0–200	No. of cases	200–400	No. of cases	400–600	No. of cases	600–800	No. of cases	Over 800
Soils										
Farmyard manured	2	·000245	2	·000140	7	·000126	3	·000098	33	·000039
Unmanured	12	·000149	9	·000053	1	·000055	2	·000049	2	·000049
$NaNO_3$	24	·000365	10	·000058	5	·000062	1	·000029	—	—
$(NH_4)_2SO_4$	16	·000190	7	·000091	—	—	—	—	1	·000041
Sands										
Peptone	4	·000092	11	·000080	9	·000042	5	·000105	5	·000063
Soil extract	28	·000259	8	·000068	5	·000058	—	—	7	·000017
$NaNO_3$	14	·000558	1	·000112	—	—	—	—	—	—
$(NH_4)_2SO_4$	19	·000790	—	—	—	—	—	—	—	—

Table VI.

Amount of carbon dioxide in grammes given off from 400 *gm. of different media by varying numbers of bacteria.*

		Numbers of bacteria in millions per gramme									
		No. of cases	0–200	No. of cases	200–400	No. of cases	400–600	No. of cases	600–800	No. of cases	Over 800
Soils											
Farmyard manured	Bacteria alone	—	—	—	—	—	—	1	·0228	32	·0216
	Bacteria and amoebae	—	—	—	—	1	·0256	1	·0404	31	·0240
Unmanured	Bacteria alone	11	·0072	9	·0056	1	·0100	1	·0128	2	·0068
	Bacteria and amoebae	11	·0068	7	·0068	4	·0104	—	—	2	·0440
$NaNO_3$	Bacteria alone	13	·0072	8	·0052	4	·0072	1	·0092	—	—
	Bacteria and amoebae	15	·0068	8	·0072	—	—	—	—	2	·0076
$(NH_4)_2SO_4$	Bacteria alone	16	·0104	4	·0112	—	—	—	—	1	·0160
	Bacteria and amoebae	17	·0100	7	·0112	1	·0072	—	—	1	·0152
Sands											
Peptone	Bacteria alone	4	·0056	11	·0084	9	·0088	5	·0272	5	·0252
	Bacteria and amoebae	14	·0076	9	·0136	6	·0160	2	·0112	2	·0300
Soil extract	Bacteria alone	27	·0052	8	·0072	5	·0116	—	—	3	·0104
	Bacteria and amoebae	20	·0044	11	·0172	3	·0080	1	·0068	8	·0064
$NaNO_3$	Bacteria alone	20	·0040	1	·0096	—	—	—	—	—	—
	Bacteria and amoebae	20	·0088	—	—	—	—	—	—	—	—
$(NH_4)_2SO_4$	Bacteria alone	20	·0036	—	—	—	—	—	—	—	—
	Bacteria and amoebae	19	·0060	—	—	—	—	—	—	—	—

medium, it is seen that the fewer bacteria there are present the more efficient each one becomes. Information as to the influence of the medium on the efficiency of the bacteria can be obtained by considering the actual amount of carbon dioxide given off by a constant volume of medium with the same numbers of bacteria. From Table VI, though the distribution is irregular, it would appear that although an increased population gives off an increased amount of carbon dioxide on any one medium, yet the bacteria produce roughly the same amount of carbon dioxide, irrespective of the medium. The different media are unable to support the same sized populations, since in the soils the unmanured and those with minerals rarely have a density of over 400 millions per gm., while the farmyard manured are almost entirely above 800 millions. This is also shown, though in a lesser degree, by the sand experiments, where peptone supports a larger population than the others.

Effect of amoebae.

In both sands and soils the presence of amoebae has the usual result of lowering the numbers of bacteria, but the effect upon carbon dioxide production is not so simple. In Table II the correlation between carbon dioxide production and bacterial numbers, when amoebae are present, is not so sharp as when bacterial cultures alone are considered; thus on soils there is no correlation in the unmanured and minerals, and in sand the same obtains for peptone and soil extract, but the ammonium sulphate and nitrate of soda sand cultures give a χ^2 over 4 and the farmyard manured soils one of 3·9. Such divergent results are only to be expected when it is remembered that the amount of carbon dioxide given off by amoebic respiration would often be sufficiently great to mask the direct effect from carbohydrate decomposition; and that this latter is conditioned by the continual fluctuations between the numbers of bacteria and amoebae. When the action of the amoebae on the amount of carbon dioxide produced is considered it is found that, in the case of sand cultures, amoebae decrease the amount of carbon dioxide given off when peptone is used, but increase the amount in the case of soil extract and of the mineral solutions with glucose (Figs. 1 and 2). When comparing each 24-hour period for the cultures, with or without amoebae, in soil extract, on 76 per cent. of the occasions more carbon dioxide was produced when amoebae were there; in the case of mineral salts and glucose there were 66 per cent. of times when the carbon dioxide was higher with amoebae, but in the case of peptone on 67 per cent. of the occasions the carbon dioxide was less in the presence of amoebae.

If the exceptions are examined the numbers are found to be even more significantly different than appears from the figures quoted. For instance in the soil extract cultures there are nine occasions when less carbon dioxide is produced where amoebae are present, but on five of these the bacteria are lower than is the case in the cultures of bacteria alone and the numbers of amoebae are negligible, and the same holds good for six out of the fourteen exceptions for the cultures with mineral salts and glucose. In the peptone experiments there are twelve cases where the amoebic cultures give more carbon dioxide than do the purely bacterial ones. In five of these the bacterial numbers are not significantly different in the two series of cultures, but the numbers of amoebae are very high, and the amoebic respiration might well bring up the output of carbon dioxide; in one case the bacteria were distinctly higher in the amoebic cultures and in two others the bacterial numbers were higher and the amoebae were also present in large numbers. Excluding therefore those which are explicable as shown above, the percentages of exceptions are reduced to 11, 20 and 10 per cent. in the case of soil extract, minerals and peptone respectively.

In soils the results are not so clear cut; in the case of the farmyard manured soil where the expectation is that less carbon dioxide would be produced in the presence of amoebae, as is the case with peptone in sand, in actual fact this only occurred on 47 per cent. of the occasions, and out of the seventeen exceptions only seven could be explained as being due to the numbers of bacteria being higher in the series with amoebae. The unmanured soil fell more into line with the sand treated with soil extract, in that the carbon dioxide produced was greater in 64 per cent. of the cases where amoebae were present, but of the ten exceptions only one could be due to the small numbers of both bacteria and amoebae in the amoebic cultures. The soils from the ammonium sulphate and nitrate of soda plots were again unsatisfactory, since only 50 per cent. of the cases gave higher carbon dioxide in the presence of amoebae, and of the fourteen exceptions only three could be accounted for by the low numbers of bacteria and amoebae in these cultures.

In view of the complex nature of soil compared with the simple inoculated sands it would be expected that the results would not be so clear cut; further work is therefore contemplated on soils together with experiments testing the amount of carbon dioxide which can be ascribed to amoebic and bacterial respiration as distinct from that produced by carbohydrate decomposition.

SUMMARY.

Experiments are described on carbon dioxide production from soil and sand cultures containing a species of bacterium with and without amoebae. The following results were obtained:

1. Carbon dioxide production and bacterial numbers are correlated provided that amoebae are not present, or are present in very small numbers.

2. The bacteria are more efficient as producers of carbon dioxide when their numbers are not rising and less efficient when their numbers are increasing. This does not hold for young cultures. Also each bacterium becomes less efficient as the density of the population increases.

3. The amoebae cause a decrease in carbon dioxide production in sands containing peptone, but an increase in sands containing mineral salt solution with glucose or soil extract

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OBITUARY NOTICE

Prof. J. RITZEMA BOS

1850–1928

Honorary Member of the Association of Economic Biologists.

(With Plate XXI.)

J. Ritzema Bos, one of the most distinguished pioneers in applied biological science, was born on July 27th, 1850, at Gröningen, where also he received his elementary and secondary education. At the University of his native town, besides studying in particular Zoology and Botany, he attended a course in Agriculture under H. C. van Hall. While still a student, he gained a University prize with a paper on "The Fauna of the Dutch Islands in the North Sea," and similar investigations formed the subject of his degree thesis, "Crustacea-hedriophthalmata of the Netherlands" in 1874. Before taking his doctorate, he had already (1869) been appointed teacher in Zoology at the Agricultural School in Gröningen.

Ritzema Bos' subsequent professional life may be briefly outlined. In 1871 he became a teacher in Zoology at the Agricultural Secondary School at Warffum (Friesland). Two years later we find him in a similar post at Wageningen and when, in 1876, a higher agricultural school was founded there, he was again appointed to teach his favourite subject. Nearly twenty years of research and teaching followed. The results were apparent when, in 1895, he was made head of the new Phytopathological laboratory—the "Willy Commelin Scholten"—in Amsterdam. This was a strenuous period in Ritzema Bos' life, as he was also assistant-professor in the University and continued to discharge his duties at Wageningen.

It was not, however, till four years later that Prof. Ritzema Bos' ideas of the rôle of Applied Biology in Agriculture, Forestry and Horticulture began to have full effect with the founding in Amsterdam of a national Phytopathological Service—an institute designed not merely to diagnose and prescribe, but, above all, to see that the advice given is efficiently carried out. Such a service was fittingly enough inaugurated in a land which, for over three centuries, had been noted for its intensive cultivation. At the present day, *e.g.* two heavy crops of cabbage per field

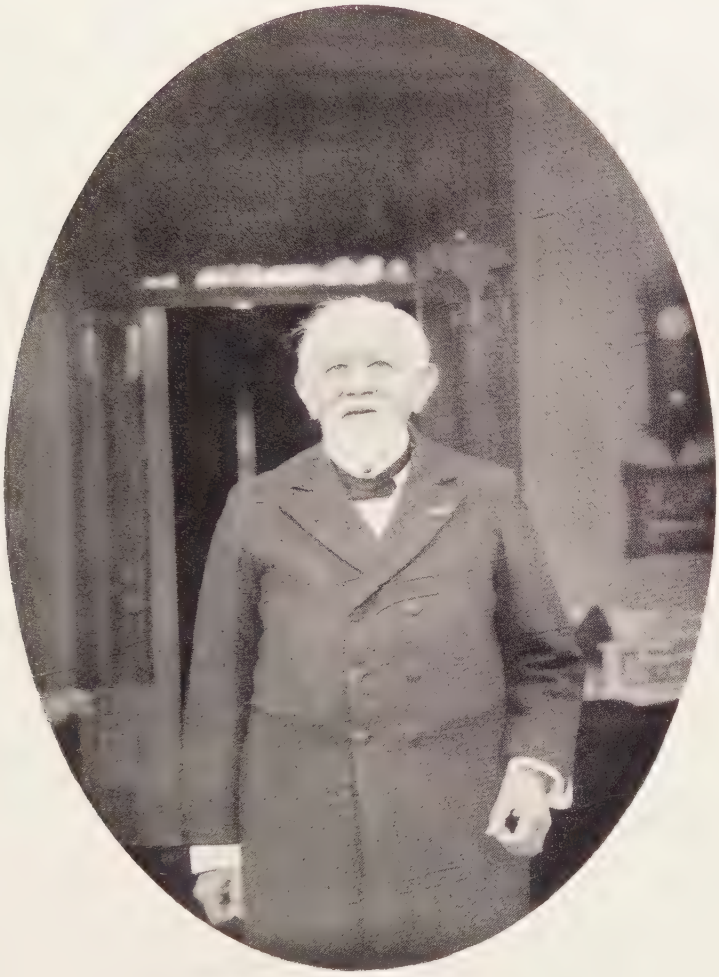
is the rule, and the average yield of potatoes is 241 hectolitres per hectare, while exceptionally 329 hectolitres may be raised. Of this new service Ritzema Bos became head (1895)—his whole career since 1869 fitted him unquestionably for the post—and his impress on it has been deep. At present the service, with a personnel of sixty official members, in conjunction with three hundred voluntary co-operators (for the most part prominent local agriculturists or horticulturists holding the diploma of a recognised school) carries out a vast and varied campaign against disease in plants. The work is divided into several sections, embracing research into cases submitted (about two hundred per month) and the giving of advice; propaganda—with two branches dealing with diseases in horticulture and agriculture respectively; ornithology—concerned with aviculture in woods and orchards. (An interesting and original departure, *e.g.* nesting boxes and breeding holes are multiplied and the results tabulated; effects of egg-gathering on gulls and plovers studied; general investigation of bird food and special research carried out on such species as the rook, starling and plover.) There are also sections (*a*) for museums, collections and exhibitions, (*b*) inspection and the enforcing of legislation, (*c*) administration and statistics.

In 1906 Ritzema Bos became Head of the newly founded Institute for Plant Diseases at Wageningen, and lecturer in Phytopathology in the higher agricultural school. In 1918 this school was raised to the status of a University of Agriculture and Dr Ritzema Bos became a professor. When, in 1919, the Phytopathological Service was separated from the Phytopathological Institute, he retired from the headship of the former and next year, at the age of seventy, gave up official work. After his retirement, however, he still acted as adviser to the Phytopathological Service.

Although Dr Ritzema Bos' interests lay, at first, in animal physiology, he soon devoted himself entirely to research in phytopathology, and there especially to helminthology and economic entomology.

In 1891 he founded the Nederlandsche Phytopathologische Vereeniging in conjunction with Prof. Hugo de Vries. He was president of this Society till his death. From 1895 he edited the *Tijdschrift voor Plantenziekten*. With the late F. B. Löhnis and the late Dr P. van Hoek and L. A. Springer, he edited the *Geillustreerde land- en tuinbouwbibliotheek* (Illustrated Agricultural and Horticultural Library).

Amongst his principal contributions to biological literature are the following: "L'Anguille de la Tige (*Tylenchus devastatrix* Kühn), et les maladies des plantes dues à ce Nématode," *Archives Teyler*, Harlem,



PROFESSOR J. RITZEMA BOS

1888–92, Sér. II, Tome III, and, in the same series (Tome II, 2, 1885) appeared his monograph, "La Mouche du Narcisse (*Merodon equestris* F.), ses métamorphoses, ses mœurs, les dégâts causés par les larves et les moyens proposés pour la détruire." His well-known *Zoologie für Landwirte* had a great vogue on the continent, attaining to a seventh edition in 1923. Translated into English by R. A. Davis under the title *Agricultural Zoology* (1894), the book was welcomed and attained a second edition (1900). Another major work was *Tierische Schädlinge und Nutzlinge*, 1891. In the Illustrated Agricultural and Horticultural Library, above referred to, Dr Ritzema Bos contributed longer articles on the anatomy and physiology of domestic animals; diseases and injuries of fruit trees; diseases of crops, etc. For his students also, he produced text-books on general and agricultural zoology.

From 1870 onwards Ritzema Bos published very many notes, observations and shorter articles which will be found mainly in the following serials: *Tijdschrift voor Plantenziekten*; *Tijdschrift voor Entomologie*; *Archives Teyler*, Harlem; *Die landwirtschaftlichenversuchtstationen*; *Zeitschrift für Pflanzenkrankheiten*; *Biologisches Zentralblatt*; *Zeitschrift für Bacteriologie und Parasitenkunde*.

Beyond his native land, Dr Ritzema Bos' great experience and special knowledge were widely recognised. He was one of our oldest honorary members, and had received a similar distinction from the American Association of Economic Entomologists, and the Deutsche Gesellschaft für Angewandte Entomologie. In 1899 he was honoured with a knighthood (Knight of the Dutch Lion) for his services to the State.

In the course of a long professional career, he travelled extensively, sometimes for the purpose of research, as *e.g.* when in 1899 he studied the San José scale, or again when, as an official delegate, he returned to the United States (1921) to discuss the conditions of the import of Dutch bulbs, shrubs and trees. Ritzema Bos' position as a writer and organiser is an assured one. A man of rare and practical vision he laid the foundations of applied biology in Holland with a firm hand. By his students, too, he will always be remembered for his unselfish helpfulness and the constant inspiration of his indefatigable work.

(I am greatly indebted to my friend, Dr J. Betrem, Entomological Laboratory, Wageningen, for many of the details of this short notice—particularly as regards Dr Ritzema Bos' early life. The accompanying photograph, taken in May 1924, is by Dr Roepke.)

J. WATERSTON.

REVIEWS

Plant Diseases. By F. T. Brooks. Oxford University Press, 1928. Pp. vi + 386. 62 Illustrations. Price 21*s.* net.

Since the publication in 1910 of Massee's *Diseases of Cultivated Plants and Trees* there has appeared in this country no general account of the diseases of British crop plants. "The present book endeavours to supply this deficiency. In it the author has also attempted to include an outline of our present knowledge of important plant diseases in other parts of the Empire and in other countries of the world." This wider field has of course been covered by numerous treatises published in America, Germany and elsewhere during recent years. The scope of the present work is briefly as follows.

Chapter I is a general introduction to the subject, dealing shortly with economic losses, etiology, symptoms and spread of disease, the influence of environmental conditions and disease control. Chapter II is a summary treatment of non-parasitic diseases. Chapters III–VI are devoted respectively to diseases caused by Viruses, Bacteria, Actinomycetes and Myxomycetes. Chapter VII is introductory to the main part of the book and contains a brief general account of the fungi with a synoptic scheme of classification. Chapters VIII–XX are given to diseases caused by fungi. Beginning with Phycomycetes, the author works through the Ascomycetes, Basidiomycetes and Deuteromycetes. In turn each group is briefly described, then the alliances and the genera within the alliances and finally the diseases caused by particular species. The scientific name of the fungus is followed by the common name of the disease recommended by the British Mycological Society, a synopsis of the fungus and an account of the host plants, symptoms, distribution and treatment of the disease. In chapter XXI the algal disease of tea is described and the last chapter contains a brief account of some common fungicides and is followed by an index. To each chapter is appended a selected list of references.

The sixty-two illustrations deserve comment, favourable in that they are all original, unfavourable in that some of them are of mediocre quality. Further it is not easy to understand the rationale of the author's choice or apportioning of illustrations for they seem to bear little relation to the comparative importance of the several diseases or groups of parasites.

Whilst reading the volume one is not infrequently arrested by points on which opinions might diverge but these usually concern detailed matters and merely emphasise the "up-to-dateness" and general excellence of the work and the relatively enormous amount of matter the author has compacted within it.

The criticism one might make of this book is more fundamental, namely, that it presents what many pathologists would consider a wrong balance of values, and as the book is probably destined to become the accepted English text this is a matter of real concern. The work is entitled *Plant Diseases*, with no qualifying sub-title, and the preface states that "The author has had a good deal of experience in training men to serve as Plant Pathologists at home and abroad, and his book is designed particularly to assist such students and others who are carrying out investigations on Plant Diseases. It is hoped also that the book will be useful to the general botanist, to students of Agriculture, Horticulture, and Forestry, and to those cultivators of the soil who take an enlightened interest in the crops they grow." The work therefore represents the author's matured judgment of the comparative importance of the several regions of plant pathology and of the different diseases of Empire crops. The minor criticism may be illustrated by a single example. It is easy to sympathise with Mr Brooks' tendency to overflow on silver-leaf disease, but it hardly seems justifiable to devote six pages to this one malady and only twelve pages to either the specific virus diseases or the bacterial diseases. The larger criticism however involves the orientation of the author's view-point towards the science of plant pathology. Diseases

of plants are usually divided into two groups—"non-parasitic diseases" and "parasitic diseases." Mr Brooks accepts this division but, merely stating that "In this book it is not possible to give a full account of non-parasitic diseases, and only a few of the commoner maladies of this kind will be discussed," he waives them aside in eight pages with no illustrations. It might be thought equally impossible to give a full account of parasitic diseases yet these receive 340 pages and 62 illustrations. It is almost as if the functional diseases were practically omitted from a medical text-book, their exclusion being justified by the author on the ground that it was not possible to give a full account of them, or as if in the present work Mr Brooks had reversed his procedure and had discussed the non-parasitic diseases in 340 pages and dismissed in eight pages the diseases caused by viruses, bacteria and fungi. The difference in view-point may be appreciated if one compares the present volume with standard works which have recently appeared in Germany, France and the United States of America, for example Sorauer's *Handbuch der Pflanzenkrankheiten*, Delacroix and Maublanc's *Maladies des Plants Cultivées* and Heald's *Manual of Plant Diseases*.

To justify the title of "Plant Diseases" and give a right perspective to those for whom the author states that he has written the book, one may hope that he will regard this as volume one and produce an equally useful second volume in which the non-parasitic diseases receive due consideration.

The book is dedicated to Marshall Ward, of whom a fine portrait serves as frontispiece, and it is well produced but misprints have been noted on pages 50, 205, 246, 361 and 371, and some word such as "only" seems to have been omitted from line 3 on page 263.

As a handy compendium of the fungus diseases of plants the work is one of the most useful additions to the literature of plant pathology that has been published for some time and it will be found indispensable by all British students and practitioners of the science.

WILLIAM B. BRIERLEY.

Laboratory Manual of General Microbiology: with special reference to the micro-organisms of the Soil. By E. B. Fred and S. A. Waksman. McGraw-Hill Publishing Co., Ltd., 1928. Pp. v + 145. 19 Figs. Price 10*s*. net.

In America particularly, and due in no little part to the activities of the two authors, a scientific synthesis known as "General Microbiology" is rapidly developing. It is concerned with the lives and activities of the simpler algae, lower fungi, bacteria and protozoa as these occur in diseased states, industrial processes, soil, sewage and other economic environments. The American synthesis received general statement in the valuable compilation edited by C. E. Marshall some years ago but since then considerable advances have been made and particular aspects, such as soil microbiology or the microbiology of disease, have become prominent scientific issues. The general science is taking an important place in the curriculum of agricultural colleges, technical institutes, etc. and is gathering about itself a considerable special literature. The present book belongs to this and has been designed specially for students of soil-biology. It is divided into four parts. The first contains general directions for the making of culture media and details of one hundred and eleven different media for all sorts of micro-organisms. Part II is devoted to methods of staining and is not quite so good as it might have been. Part III is a very useful account of qualitative and quantitative methods of analysis and is written simply and practically. Part IV consists of 58 laboratory exercises covering generally the study of micro-organisms in the soil. It also contains a classified list of the more important books of reference and the addresses of laboratories where pure cultures may be obtained.

Throughout, special attention is paid to the physiology of the micro-organisms, more especially perhaps to their fermentation processes, in an attempt to portray not only their qualitative but also their quantitative relationships. No attempt is made to mention all the biological or chemical methods employed in microbiological

studies and it is in the selection of one method rather than another that scope is, perhaps, afforded for criticism. On the whole the authors seem to have been extremely judicious in their selection which must have been a difficult task.

The book is well produced and strongly bound, a quality very desirable but often overlooked in a volume whose fate it is to lie about on working benches. It will be found very handy in all laboratories where micro-organisms are studied.

WILLIAM B. BRIERLEY.

The Principles of Applied Zoology. By Robert A. Wardle. Longmans, Green & Co., 1929. Pp. xii + 427. 55 Illustrations. Price 21*s.* net.

This book attempts to embrace in one compass the whole of the diverse aspects of zoology in relation to man and his operations. We cannot recollect a similar kind of book covering so wide a field—in fact so wide is the field that it is usually catered for in a number of separate treatises. In attempting this task Prof. Wardle has at least shown how numerous are the phases of human activity which require expert zoological knowledge. At the same time he has compressed a large amount of information in a relatively small compass.

Part I, which consists of twelve chapters, deals with medical and veterinary zoology. Four chapters are concerned with Protozoa, their structure, classification and distribution, followed by accounts of the enteric and haematophilous forms. A similar number of chapters are given over to Helminthes and likewise to Arthropoda. Part II also comprises twelve chapters and is concerned with agricultural and horticultural zoology. The first chapter discusses in general terms the fauna of the soil. Then follow six chapters on insect pests and a chapter each on Vermin Repression, Bird Encouragement, Animal Domestication, Types and Breeds of Farm Animals and Livestock Breeding. Part III is concerned with animal industries including bee-keeping, silk and lac culture, fisheries, whaling and sealing, fur-bearing animals and the trades concerned and animal conservation. There are ten chapters on these subjects and they extend over less than 100 pages. Part IV of the book is composed of a classified bibliography and an index.

Viewing the book as a whole we can say definitely that Prof. Wardle has surveyed his subject well. The real trouble is that he has set himself the task of taking cognisance of the whole realm of applied zoology within a compass of about 400 rather small pages. He obviously has had to cut his cloth according to the amount available and too much must not be expected for that reason. He has, however, succeeded in providing an extraordinarily wide survey of his subject, based upon an extensive acquaintance with its literature. Since his volume bears the title of "Principles" it is perhaps a little anomalous to find that it is much stronger in facts and that principles rather fade into the background. In some of the chapters, for example, it is not easy for the reader to gather where the zoologist comes in and how he can assist the practical man. One of the greatest contributions of zoology to practice is the unravelling of the laws of heredity. Man is thus provided with the means by which he is enabled to improve animal breeding, whether the animals be cattle or silkworms. In apiculture we need a method of scientifically controlled mating of the queen bee, and, with the possibilities of artificial insemination, that desideratum may already be in sight. With noxious insects the application of insecticides has long held the field, but we are beginning to realise the importance of studying those factors which conduce to infestations. A notable advance may well come from zoological studies in this direction combined with help from other sciences. Of lac culture India has a virtual monopoly, and one of the obstacles to improvement is the difficulty of eliminating parasites—and here the skilled zoologist is essential. With fisheries the big zoological problem is the study of food-fishes to a degree of exactness, as regards their behaviour and conditions of life, that will enable us to conserve our diminishing supplies: and the same applies to whaling. These are but a few examples of how the applied zoologist, utilising advances in pure science, can make himself indispensable.

We think that applied zoologists will welcome this volume as a comprehensive

outline of their subject. Its freedom from errors and the clear method of presentation commend it to the student, while the useful bibliography at the end guides the reader to abundant sources of further information.

A. D. IMMS.

Agricultural Entomology. By D. H. Robinson, B.Sc. and S. G. Jary, B.A. London: Duckworth, 1929. Pp. vii + 314. 149 Text-figures. Price 15*s.* net.

For a number of years past there has been a very evident need for a reliable up-to-date text-book of agricultural entomology and even to this day John Curtis' *Farm Insects* is still, in spite of its antiquity, the most exhaustive volume we possess. A second "Curtis" is badly required, but it demands exceptional qualifications on the part of anyone who is sufficiently public spirited to embark on its production. He must be a man endowed with breadth of outlook on both the scientific and applied aspects of his subject and, at the same time, capable of utilising to the best advantage the mass of valuable information that is yearly accumulating in continental journals and text-books.

The volume before us is of a much more elementary character than the one just envisaged. It is a students' handbook and one necessarily confined, owing to limits of space and cost, to a briefer method of treatment. The first part is devoted to an outline of the general structure, life-histories and classification of insects. The second part deals with the various insects of importance to agriculture under their respective orders, and briefly describes preventive or remedial measures for combating them. At the end of this section are two chapters dealing respectively with the principles of insect control and with the composition and application of insecticides. A number of animals, other than insects, which also concern the agriculturist are discussed in a series of short appendices. The book concludes with hints on entomological technique, a short list of literature and an index.

Of the two authors Mr D. H. Robinson is Head of the Biology Department of the Harper Adams Agricultural College and Mr S. G. Jary is an Advisory Entomologist for the Southern Province under the Ministry of Agriculture scheme. Viewed as a whole the book they have written is up to date and should meet the demand it is evidently intended to cater for. It gives the essential information the student requires and, if supplemented by adequate laboratory and field training, the candidate should then be sufficiently well equipped to fulfil the requirements in entomology for an agricultural degree or diploma. Its clear method of expression, excellent type and good text-figures make it an attractive volume. If the practical farmer or grower can be induced to read it, he will find himself tolerably well informed of the various pests he has to contend with; he will also learn about some of the control measures which he is, as a rule, much more interested in. As many garden pests are also included it should further appeal to a proportion of the general public.

It is usually considered to be a reviewer's function to point out errors and omissions, even if it is only to prove that he has actually read the book before him, but they often tend to prejudice a book to a disproportionate degree. It is necessary, however, to mention that there are slight errors on pp. 42 and 43; the legends of Figs. 70 and 72 have been inadvertently transposed; on p. 92 Membracidae should read Cercopidae; and in the account of the Diptera (p. 193) the authors have misunderstood the significance of the frontal lunule. Such points will doubtless be corrected in the event of a new edition being called for. At the same time the authors would be well advised to give more definite information with reference to the control of the apple Capsid (p. 93), Millipedes (p. 285) and some other pests. With regard to Leather Jackets (p. 200) it appears that they are unaware that the Protection of Animals Act of 1911 was amended in 1927 so as to allow under "reasonable precautions" of the application of such methods as poison baits which are a valuable control measure.

A. D. IMMS.

PROCEEDINGS OF THE ASSOCIATION OF ECONOMIC BIOLOGISTS

Ordinary Meeting of the Association held at 2.30 p.m. on March 15th in the Imperial College of Science and Technology, London. The President, Dr E. J. Butler, C.I.E., F.R.S., in the Chair.

I. "Some Agricultural Problems in Australia" by Sir John Russell, F.R.S.

II. "The Commonwealth Council of Science and Industry in its relation to Agriculture" by F. L. McDougall, Esq., C.M.G.

Meeting of the Association on May 10th at the Docks of the Port of London Authority. London and St Catherine's Docks—Cacao, Dried Fruit, Spices, Ivory, Wool, Essential Oils and Wines. Royal Victoria and Albert and King George V Docks—Methods of handling and storage.

Field Meeting of the Association at Cambridge on Friday, June 21st, and Saturday, June 22nd. Visits to University Farm, Potato Virus Research Station, Low Temperature Research Station and Wicken Fen. Association Dinner in Christ's College.

INTERNATIONAL CONFERENCE FOR PHYTOPATHOLOGY AND ECONOMIC ENTOMOLOGY—HOLLAND, 1923.

SECOND NOTICE ON ERIKSSON PRIZES.

The Committee beg to announce that two prizes are hereby offered for the two best Memoirs, giving an account of new and original work on the two following subjects respectively:

(1) Investigations on Rust (Uredineae) Diseases of Cereals (Wheat, Oats, Barley or Rye).

(2) Investigations on the rôle played by insects or other invertebrates in the transmission or initiation of Virus Disease in Plants.

The value of each prize will be 1000 Swedish crowns.

Competitors may be of any nationality.

Three typewritten copies of each Memoir must be submitted. They may be written in any one of the three languages, English, French or German.

Memoirs must reach the Secretary on or before May 1st, 1930.

The author's name must not appear on the Memoir itself, but each Memoir must be marked with a pseudonym or a motto and the full name and address of the author must accompany the Memoir, being enclosed in a sealed envelope bearing on its outside the same pseudonym or motto as is given on the Memoir.

The adjudication of the rust prize will rest with a Jury, consisting of Prof. Dr Jacob Eriksson, Prof. Dr E. C. Stakman and Prof. Ét. Foëx.

The adjudication of the virus prize will rest with a Jury, consisting of Prof. Dr H. M. Quanjer, Dr A. D. Imms and Dr L. O. Kunkel.

The decisions of these Juries will be final, and will be announced at the Fifth International Botanical Congress, to be held in Cambridge (England) from August 16th to 30th, 1930. The copyright of the prize Memoirs will become the property of the Committee, who will endeavour to secure publication of them in a suitable existing periodical or, failing that, procure publication in some other way. Other Memoirs will be returned to their respective authors.

The Committee reserve the right to withhold the prizes should none of the Memoirs submitted be deemed of sufficient merit by the respective Juries.

Further particulars, if required, may be obtained on application to the Secretary.

For the International Committee for Phyto-pathology and Economic Entomology

(Signed) T. A. C. Schoevers, *Secretary*,
Nassauweg 28, Wageningen, Holland.